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201-16569B

Revised Test Plan for Ketone Bottoms (KB4/KB3)

Ketone Bottoms (KB4/KB3)

Eastman Chemical Company

Submitted to the EPA under the High Production Volume (HPV) Challenge Program by:

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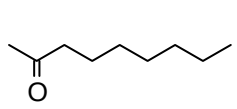
The HPV Challenge Program
Test Plan for Ketone Bottoms (KB4/KB3)

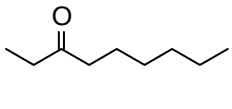
1 IDENTITY OF SUBSTANCES

Ketone Bottoms 4 (KB4)

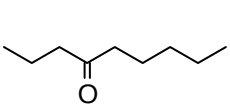
Nonanone isomers and structurally related isomers (17.2%)



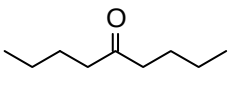
2-nonanone
CAS No. 821-55-6



3-nonanone
CAS No. 925-78-0

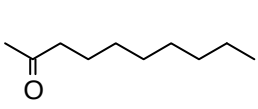


4-nonanone
CAS No. 4485-09-0

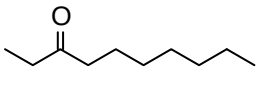


5-nonanone
CAS No. 502-56-7

Decanone isomers and structurally related isomers (13.3%)



2-decanone
CAS No. 693-54-9



3-decanone
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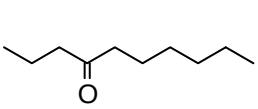
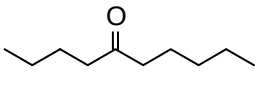
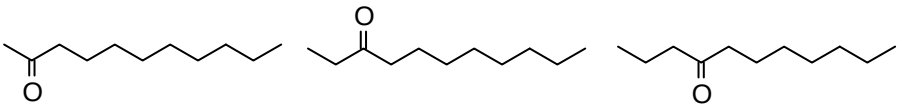


4-decanone
CAS No. 624-16-8



5-decanone
CAS No. 820-29-1

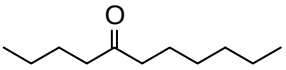
Undecanone isomers and structurally related isomers (17.8%)



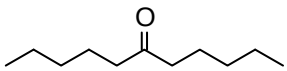
2-undecanone
CAS No. 112-12-9

3-undecanone
CAS No. 2216-87-7

4-undecanone
CAS No. 14476-37-0

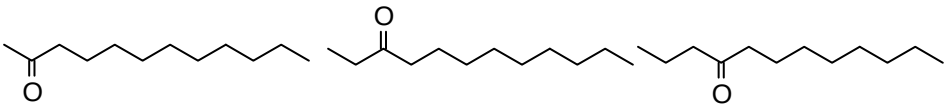


5-undecanone
CAS No. 33083-83-9



6-undecanone
CAS No. 927-49-1

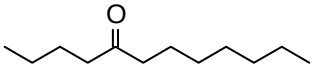
Dodecanone isomers and structurally related isomers (3.4%)



2-dodecanone
CAS No. 6175-49-1

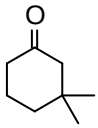
3-dodecanone
CAS No. 1534-27-6

4-dodecanone
CAS No. 6137-26-4



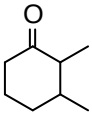
5-dodecanone
CAS No. 19780-10-0

Dimethylcyclohexanone isomers (10.8%)



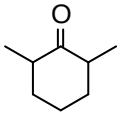
3,3-dimethylcyclo hexanone

CAS No.2979-19-3



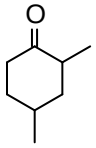
2,3-dimethylcyclo hexanone

CAS No.13395-76-1



2,6-dimethylcyclo hexanone

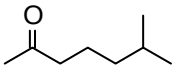
CAS No.2816-57-1



2,4-dimethylcyclo hexanone

CAS No.823-55-2

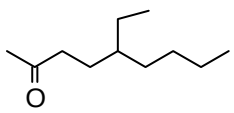
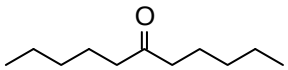
6-Methyl-2-heptanone (2.5%)



6-methyl-2-heptanone

CAS No.928-68-7

Ketone Bottoms 3 (KB3)

<table>
<tr><td>Undecanone isomers (approx. 35%)
 
5-ethyl-2-nonanone
CAS No. 5440-89-1
17.7%
+
 
6-undecanone
CAS No. 927-49-1
14.2%
+ other C_{11} ketones
(e.g., 3-butyl-2-heptanone)</td></tr>
<tr><td>Cycloheanone/cyclohexyl derivatives (approximately 16%)
3-Methyl-5-propylcyclohexanone (CAS No. 67662-89-0): 4.5%
3-Methyl-5-propylcyclohexenone: 3.1%
3-Methyl-4-butylidene-5-propyl-2-cyclohexenone: 3.4%
Other cyclohexyl derivatives: 5.3%</td></tr>
<tr><td>Mixture of 2-pentadecanone (CAS No. 2345-28-0), 6-pentadecanone (CAS No. 1001-45-2), and 8-pentadecanone (CAS NO. 818-23-5) (approximately 5%)</td></tr>
</table>

2 CATEGORY ANALYSIS

2.1 Introduction

In November of 1999, Eastman Chemical Company (Eastman) committed to participate in the Chemical "Right-to-Know" Program. As part of this commitment, Eastman is committed to assembling and reviewing available test data, developing and providing a test plan for the ketone mixtures recognized as Ketone Bottoms 4 and Ketone Bottoms 3 (KB4 and KB3), and, where needed, conducting additional testing. The CAS No. for KB4 and KB3 is 68990-20-5. The test plan and robust summaries presented are the first phase of Eastman's commitment to the Chemical "Right-to-Know" Program.

Background Information

The chemical category designated "Ketone Bottoms 4 and Ketones 3 (KB4/KB3)" consists of a continually varying mixture of linear- and branched-chain aliphatic ketones possessing carbon chain lengths from C_9 to C_{12}. These ketones account for approximately 50% of the mixture. The remaining constituents of known structures in KB4 include a mixture of dimethylcyclohexanones (10.8%) and 6-methyl-2-heptanone (2.5%). Based on the boiling point range for the distillate KB4 and the method of production, it is anticipated that the portion of KB4 of unknown composition is a complex mixture of ketones of similar molecular weight and structure. Similar to KB4, "Ketone Bottoms 3 (KB3)" is a mixture of linear- and branched-chain aliphatic and alkyl-substituted cyclohexanone derivates ketones. Isomers of undecanone constitute more than 35% of KB3 with smaller amounts of cyclohexanone (11%) and pentadecanone derivatives (5%). Other ketones in KB3 contain from 9 to 15 carbons.

Many of the constituents of KB4 and KB3 are common components of traditional foods occurring in fruits, cheese, meats, and some vegetables [CIVO-TNO, 1999]. 2-Nonanone, 3-nonanone, 2-undecanone, 2-tridecanone, 2-pentadecanone, a variety of isomeric

branched-chain ketones and methyl-substituted cyclohexanones are currently recognized by the U.S. Food and Drug Administration (FDA) as GRAS ("generally regarded as safe") for their intended use as flavoring substances [Hall and Oser, 1965]. These ketones have also been evaluated by The Joint Expert Committee on Food Additives (JECFA) as part of the World Health Organization (WHO). JECFA has reviewed numerous constituents of the KB4 and KB3 mixtures and approved them for use in flavors at current levels of intake [JECFA, 1999, 2003]. Quantitative natural occurrence data indicate that oral intake of these substances occurs predominantly from consumption of food in which they occur naturally [Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985]. Based on the long term recognition of many of these ketones as GRAS in the United States, their recognition as safe for addition to food by JECFA, and their widespread natural occurrence in food, it is recommended that no additional studies are required to meet human health endpoints in the High Production Volume (HPV) Chemical "Right to Know" Program.

2.2 Structural Classification

The chemicals of known structure in the KB4 and KB3 mixtures exhibit common skeletal and functional group features. Aliphatic linear and branched-chain substances in KB4 and KB3 are homologues with chain length from 9 to 15 carbons. Other constituents of the mixture are alkyl-substituted cyclohexanones or simple branched-chain aliphatic ketones. All contain a single ketone functional group. The stereochemistry of the alkyl-substituted cyclohexanones is not defined. Therefore all possible *cis* and *trans* isomers are anticipated. Primarily, KB4 and KB3 are composed of a mixture of higher molecular weight (greater than C_9) saturated aliphatic ketones and alkyl-substituted cyclohexanones.

2.3 Industrial Production

Eastman Chemical Company produces two products that originate as by-products from the manufacture of low molecular weight (C6-C7) straight-chain and branched aliphatic ketones and during the production of a C5 straight-chain aliphatic ketone. These products

are a high-boiling fraction removed from the primary products by distillation as an underflow stream and are commonly referred to as "ketone bottoms" (KB3 and KB4). The distillation process is controlled so as to limit the concentration of low-boiling components in this stream. In addition, all manufacturing processes are considered as closed processes. The material is piped to on-site storage tanks and is than transported by tanker truck or railcar to other industrial locations.

2.4 Chemical Reactivity and Metabolism

2.4.1 Aliphatic Linear Ketones

Aliphatic ketones in this group are rapidly absorbed through the gastrointestinal tract and eliminated from the blood. Peak blood levels are normally obtained within 1-2 hours after dosing [Lehman *et al*., 1945; Nordmann *et al.*, 1973; Bonte *et al.*, 1981]. The corresponding secondary alcohols (*i.e*., 2-nonanol is the corresponding secondary alcohol of 2-nonanone) are also rapidly absorbed and readily converted to the corresponding ketone *in vivo*. Although ketones and secondary alcohols are readily interconverted in animals, reduction of the ketones by cytosolic carbonyl reductases [Felsted and Bachur, 1980] is favored yielding the corresponding secondary alcohols that are rapidly excreted in the urine mainly as glucuronic acid conjugates [Kasper and Henton, 1982; Gry, 1999].

In the case of a methyl ketone, the terminal methyl group may undergo oxidation, eventually yielding *alpha*-ketoacid that is the substrate for further cleavage and oxidation in the fatty acid pathway and citric acid cycle [Wouters and Speijers, 1999]. Therefore, a substance such as 2-nonanone is anticipated to be either reduced to 2-nonanol and excreted as the glucuronic acid conjugate or undergo *alpha*-oxidation and cleavage to eventually yield shorter chain acids that enter the fatty acid pathway and are eventually completely metabolized to carbon dioxide and water.

A third metabolic option, which for most higher molecular weight (MW) ketones is a minor pathway, is *omega*- and/or *omega*-1-oxidation. Although for most ketones, this oxidation yields polar metabolites that are further oxidized and/or excreted, exposure to

high levels of ketones that undergo *omega*- and/or *omega*-1-oxidation to form *gamma*-diketones (*e.g*. 2,5-hexadione) are known to exhibit a neurotoxic phenomenon known as "giant axonal swelling". This toxicity is observed at high levels of exposure and is limited to ketones that have structural features (2-hexanone, 3-heptanone, and 5-nonanone) that permit primarily *omega*-1-oxidation to yield a *gamma*-diketone. Of ketones with chain length equal to or greater than six, the effect is most pronounced for 2-hexanone. Higher MW ketones such as 3-heptanone and 5-nonanone only exhibit this effect at or near the lethal dose levels. However, because this is an intoxication pathway, it will be discussed in detail with emphasis on its relationship to the only ketone in KB4 that could possibly show this phenomenon, 5-nonanone.

2.4.1.1 Reduction of Aliphatic Linear Ketones

In studies limited to the identification of urinary glucuronide metabolites, relatively high single dose levels of a homologous series of aliphatic secondary alcohols and ketones were administered individually by gavage to rabbits. The urinary excretion of glucuronic acid conjugates was determined after 24 hours [Kamil *et al.*, 1953]. The substances, dose levels, and average urinary output of glucuronide (%) are listed in Table 1. The results demonstrate that secondary alcohols, either administered directly or formed *via* ketone reduction, are largely excreted as glucuronic acid conjugates.

TABLE 1 - ALIPHATIC SECONDARY ALCOHOLS AND KETONES ADMINISTERED TO RABBITS VIA GAVAGE

Substance	Dose, mg/kg bw[1]	% Urinary glucuronic acid conjugate
2-pentanol	735	44.8
2-heptanone	950	41.0
2-heptanol	965	54.6
3-heptanol	965	61.9
2-octanol	1081	15.5

[1] Calculated based on dose of 25 mmole/3 kg of rabbit

2.4.1.2 omega-Oxidation of Methylketones

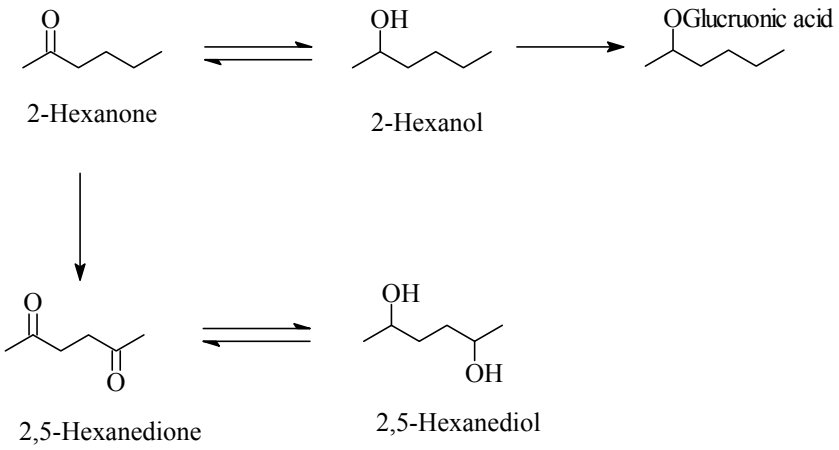
Aliphatic secondary ketones that are also methyl ketones have additional metabolic options available for the detoxication and excretion of these substances. Methyl ketones undergo *alpha*-hydroxylation and subsequent oxidation of the terminal methyl group to yield corresponding ketocarboxylic acids [Gabriel *et al.*, 1972]. The ketoacids are intermediary metabolites (*e.g. alpha*-ketoacids) that undergo oxidative decarboxylation to yield carbon dioxide and simple aliphatic carboxylic acids. The acids may be completely metabolized in the fatty acid pathway and citric acid cycle. The metabolism of *alpha*-hydroxyacids and *alpha*-ketoacids was recently reviewed [Abbott, 2000].

2.4.1.3 omega- and/or omega-1-Oxidation

Even though longer-chain aliphatic ketones (*i.e.,* carbon chain length $\geq C_5$) are primarily metabolized *via* reduction, *omega*- and/or *omega*-1-oxidation are competing pathways at high concentrations (see Figure 1). In a metabolic study, guinea pigs were given a single intraperitoneal injection of either, 450 mg 2-butanone (No. 3), 2-hexanone[1] or 5-methyl-2-hexanone[1]/kg bw in corn oil (25%). Blood serum was collected at 1, 2, 4, 6, 8, and 16 hours. Identified metabolites were then administered separately to animals at the same dose level. Analysis of blood serum revealed metabolites formed *via* ketone reduction and *omega*-1-oxidation. 2-Hexanone (2H) was metabolized by reduction to yield 2-hexanol (2OH), and *omega*-1 oxidation to yield 5-hydroxy-2-hexanone (5H2H), and then 2,5-hexanedione (25HD). The respective serum half-lives and clearance times of 5H2H (156 minutes and 8 hours) and 25HD (100 minutes and 16 hours) were approximately twice that of the parent ketone 2H (78 minutes and 6 hours) or the 2OH metabolite (72 minutes and 6 hours). When 2OH was administered to guinea pigs at the same dose level, the parent ketone 2H and *omega*-1-oxidation metabolites (5H2H, 25HD, and 2,5-hexnaediol) were identified in the serum. Intraperitoneal administration of *omega*-1-oxidation metabolites 2H5H and 25HD demonstrated that *omega*-1-oxidation metabolites were inconvertible in serum [Dietz *et al.*, 1981].

[1] A structurally related substance that is not a flavoring substance.

FIGURE 1. *OMEGA*-1-OXIDATION OF ALIPHATIC KETONES



In guinea pigs administered 5-methyl-2-hexanone, the metabolites of ketone reduction (5-methyl-2-hexanol, respectively) and *omega*-1-oxidation (5-hydroxy-5-methyl-2-hexanone, respectively) were the major serum metabolites. No diketone was detected [Dietz *et al.*, 1981]. 2-Butanone and 4-methyl-2-hexanone have been detected in the serum, urine and expired air of healthy adults [Conkle *et al.*, 1975; Zlatkis *et al.*, 1980]. A recent review of aliphatic ketones [Topping *et al*., 1994] contains a comprehensive discussion of the human pharmacokinetics and metabolism of 2-butanone.

Higher homologues also metabolize *via* carbonyl reduction and *omega*-1-oxidation. In rats, 2-heptanone (No. 8) was metabolized to 2-heptanol (No. 9) and 2,6-heptanedione, the not being a *gamma*-diketone does not exhibit neurotoxicity [Topping *et al.*, 1994].

At high dose levels, *omega*- and/or *omega*-1-oxidation of certain aliphatic ketones may yield *gamma*-diketones. 3-Heptanone may yield 2,5-hexanedione and 2,5-heptanedione while 5-nonanone may yield 2,5-nonanedione. At high levels of exposure, these diketone metabolites exhibit a peripheral neuropathy commonly recognized as "giant" axonal neuropathy [Krasavage *et al.*, 1980]. The structural features of diketone metabolites required to induce peripheral neuropathy have been well characterized. The position of the ketone functions on the aliphatic chain must be 2,5- (*i.e., gamma*). *gamma*-Diketones with terminal methyl substituents (*e.g.*, 2,5-hexanedione) and/or methyl substituents at the 3 and 4 positions exhibit the most pronounced effect. The intensity of the peripheral neuropathy depends on the size and position of alkyl substituents on the *gamma*-diketone. For example, the strongest neurotoxic effects have been observed for 3,4-dimethyl substituted 2,5-hexanedione (not a flavoring substance). When the methyl substituents are removed and the chain length is increased (*i.e.,* C_7 and greater) the neurotoxic response is significantly mitigated [Topping *et al*., 1994].

5-Nonanone is the only ketone in the KB4 mixture containing structural features that permit a neurotoxic *gamma*-diketone to form [O'Donoghue *et al.*, 1982]. In a study of commercial-grade 5-methyl-2-octanone (72.295) containing 5-nonanone (11.63%) as an impurity, rats were given 2000 mg/kg bw of the mixture orally by gavage for 90 days. After 59 days, clinical signs of neurotoxicity (tail droop and extension of hind-limb) were

observed. The treatment of rats with 2000 mg/kg bw/day of pure 5-nonanone produced signs of peripheral neuropathy. Serum analysis showed the presence of 2,5-nonanedione and the neurotoxic agent, 2,5-hexandione, presumably formed as a secondary metabolite after oxidation and chain cleavage reactions. Animals given 233 mg/kg bw of 5-nonanone for 90 days showed no signs of general or neurological toxicity. In conclusion, the presence of a butyl side-chain mitigates the neurotoxic effect of 5-nonanone, which is observed only at extremely high dose levels (greater than 2000 mg/kg bw oral dose levels).

In a study investigating the neurotoxic effects of other aliphatic ketones, 3-heptanone administered to female Wistar rats *via* drinking water at 1000 mg/kg bw/day for 120 days did not produce any evidence of neurotoxicity [Homan and Maronpot, 1978]. Male Crl rats (3/group) were exposed to atmospheres containing 700 ppm 3-heptanone for 2-20 hour and 2-16 hour periods per week. Animals were subjected to 88 exposures over 164 days (approx. 24 weeks). After the 4^{th}, 30^{th}, and 85^{th} exposure, blood serum was analyzed for 2,5-heptanedione. Maximum mean serum levels reached 10 micrograms 2,5-heptanedione/ml after 4 exposures but decreased to 6-7 micrograms 2,5-heptanedione/ml after 30 and 85 exposures. No neurotoxicity was observed [Katz *et al.*, 1980]. In a gavage study, Crl rats (2/group) were given 250, 500, 1000, 2000, or 4000 mg/kg bw of 3-heptanone (No. 10) for 5 days per week for 14 weeks. Total 48 hour urinary excretion of *gamma*-diketone (*i.e.*, 2,5-hexanedione and 2-5-heptanedione) was measured during the last week of the study and was 1.28 or 2.14 mg, respectively, for animals administered 1,000 or 2,000 mg/kg bw of 3-heptanone. No peripheral neuropathy was observed after 14 weeks at any dose level up to and including 1000 mg/kg bw. At a dose (2000 mg/kg bw) approaching the LD50 (2760 mg/kg) in rats, 3-heptanone did induce peripheral neuropathy [O'Donoghue *et al.*, 1984].

Clearly, neurotoxicity is limited to a few aliphatic ketones that must meet strict structural requirements, that is, the monoketone must possess an omega-1 position that can be oxidized via CYP-450 to yield a gamma-diketone. In addition those ketones containing alkyl substituents larger than C_1 exhibit low potential for neurotoxicity, normally at levels comparable to LD50s for the respective substance. 5-Nonanone is the only chemically

identified constituent that meets these structural requirements. However, given the size of the alkyl substituent on the ketone function (butyl), neurotoxicity occurs only at lethal levels of toxicity.

2.4.2 Summary of Metabolism and Excretion

Data demonstrate that the reviewed group of secondary alcohols and aliphatic ketones undergo efficient metabolic detoxication. Metabolism of aliphatic ketones occurs primarily *via* reduction to the corresponding secondary alcohol. Secondary alcohols are metabolized by conjugation with glucuronic acid followed by excretion primarily in the urine. Short-chain aliphatic ketones may also be metabolized *via omega*- and/or *omega*-1-oxidation, and/or they may be excreted unchanged in expired air. *omega*-Oxidation and/or *omega*-1-oxidation become competing pathways for longer-chain aliphatic ketones at high concentrations. The only identified intoxication pathway (*i.e.*, formation of a neurotoxic *gamma*-diketone) applies strictly to 5-nonanone. However, the threshold for activation of this intoxication pathway occurs at near-lethal dose levels.

2.4.3 Alkyl-substituted cyclohexanones

Cyclohexanone and alkyl-substituted cyclohexanones are rapidly absorbed through the gastrointestinal tract and rapidly eliminated from the blood. Peak blood levels are normally reached within 1-2 hours after dosing. The cyclohexanone derivative may be reduced to cyclohexanol by cytosolic carbonyl reductases. Conversely, unsubstituted or alkyl-substituted cyclohexanol is rapidly oxidized *in vivo* to the corresponding cyclohexanone derivative by alcohol dehydrogenase. Hence, just as for aliphatic ketones, cyclohexanone and cyclohexanol derivatives are inconvertible *in vivo*. Conjugation of the alcohol with glucuronic acid and excretion in the bile and urine provides the predominant pathway for metabolic detoxication and elimination of both cyclohexanol and cyclohexanone derivatives.

Male Sprague-Dawley rats were exposed to atmospheres of either 400 ppm (240 mg/kg bw) or 1,600 ppm (980 mg/kg bw) cyclohexanone for 6 hours. Twenty-four hour post-

exposure, terminal blood and urine samples show the average plasma levels of cyclohexanone and cyclohexanol for the 400 ppm and 1,600 ppm exposures were 26 and 20 micrograms/ml and 122 and 140 micrograms/ml, respectively. The total urinary excretion of cyclohexanol was at least 10 times that of cyclohexanone (16 and 15 micrograms and 143 and 264 micrograms at the 400 and 1600 ppm exposures, respectively) with 13 micrograms and 72 micrograms of conjugated cyclohexanol being excreted within 72 hours at 400 ppm and 1,600 ppm, respectively [Topping *et al*., 1994].

In another study, four rabbits were each given cyclohexanone (No. 1100) in water by gavage. Urine collected at 18 hours after dosing revealed 66% of the 248 mg/kg oral dose was excreted as the glucuronic acid conjugate of cyclohexanol [Elliott *et al.,* 1959]. The authors concluded that cyclohexanone is first reduced to cyclohexanol and then conjugated with glucuronic acid prior to excretion in the urine.

Male beagle dogs were given 284 mg/kg bw of cyclohexanone by intravenous injection daily. Cyclohexanol was detected in the plasma within 30 minutes of injection. The mean distribution and elimination half-lives of cyclohexanone and cyclohexanol are 6.6 and 81 minutes, respectively. The mean steady state volume of distribution for cyclohexanone is 2.6 L/kg and the mean total body clearance for cyclohexanone is 27.4 ml/kg/minutes. [Martis *et al.*, 1980; Koeferl *et al.*, 1981]. When 328 mg/kg bw cyclohexanol was administered by intravenous injection, it showed a plasma half-life of 99 minutes, an apparent distribution volume of 1.2 L/kg and a total body clearance of 8.8 ml/kg/minutes. Based on these data cyclohexanone and cyclohexanol are rapidly cleared from the body [Martis *et al.*, 1980]. Approximately 60% of cyclohexanone administered was recovered in the urine as a glucuronide conjugate of cyclohexanol after 24 hours. The direct renal clearance of unmodified cyclohexanone and cyclohexanol is a minor route of elimination accounting for less than 1% of administered dose. It is proposed that 74-100% of cyclohexanone is converted to cyclohexanol and further metabolized before elimination. The authors propose that some of the cyclohexanone may be expelled through the lungs [Martis *et al.*, 1980; Koeferl *et al.*, 1981].

Four men and four women volunteers were exposed to an environment containing atmospheric concentration of 101, 207, or 406 mg/cu.m of cyclohexanone for 8 hours. Urine collected at 2-hour intervals during exposure, and for 72 hours post-exposure, shows the presence of glucuronic acid conjugates of cyclohexanediol with peak excretion rate at about 16 hours post-exposure. Approximately 60% of the cyclohexanone dose is excreted within the 72-hour period [Mraz *et al.*, 1994].

An adult man ingested 100 ml of liquid adhesive containing 39% cyclohexanone. The cyclohexanone was rapidly absorbed. Plasma and urine levels of cyclohexanone and metabolites were unaffected by gastric lavage (5.5 L saline), two plasma exchanges (2.4 L each) and hemoperfusion when compared to pre-treatment values. Cyclohexanol and cyclohexanone were detected in the plasma for up to 25 hours post ingestion. Cyclohexanone levels were at the lower limit of detection; however, plasma levels of cyclohexanol were high, 220 micrograms/ml 5 hours after ingestion and decreased to 10 micrograms/ml after 20 hours. High levels of cyclohexanol glucuronide were detected in the urine for up to 48 hours. Urinary excretion of the parent ketone was described as minimal. The elimination half-life of cyclohexanone in human plasma was determined at 4.75 hours and the rate of elimination (K_e) 0.145 micrograms/ml/hour. This indicates that the mechanism of elimination in humans involves conversion of the cyclohexanone to cyclohexanol followed by conjugation with glucuronic acid [Sakata *et al.*, 1989].

Other unsubstituted alicyclic ketones (*e.g.,* cyclopentanone) are rapidly absorbed, metabolized, conjugated and excreted mainly in the urine [James and Waring, 1971]. The urine collected from rabbits orally administered 193 mg/kg bw cyclopentanone was taken and treated with *beta*-glucuronidase. The resulting analysis revealed that the major urinary component was a glucuronic acid conjugate of cyclopentanol [James and Waring, 1971].

The size, position, number, or stereochemistry of alkyl substituents on the cyclohexyl ring exerts no significant effect on the rate of absorption, metabolism and excretion of alkyl-substituted cyclohexanol or cyclohexanone derivatives. Alkyl-substituted cyclohexanones are also rapidly absorbed, reduced to the corresponding cyclohexanol

derivatives that are then conjugated with glucuronic acid and also excreted mainly in the urine. Alkyl-substituted cyclohexanols are rapidly absorbed, conjugated with glucuronic acid, and excreted mainly in the urine.

The urine of groups (6 to 10) of doe albino rabbits was pooled 24 hours after each animal received a single oral dose of 652 mg/kg bw of (±)-2-*tert*-butylcyclohexanone, 652 mg/kg bw of (±)-3-*tert*-butylcyclohexanone, or 562 mg/kg bw of 4-*tert*-butylcyclohexanone [Cheo *et al*., 1967]. The mean % of the dose excreted as the glucuronic acid is 76.5, 90, or 80% respectively.

Rabbits given oral doses of 1,750 mg/kg bw of methylcyclohexanol (mixture of isomers) or 560 mg/kg bw of methylcyclohexanone (mixture of isomers) predominantly excrete the glucuronic acid of methylcyclohexanol within the first 24 hours [Treon *et al.*, 1943a]. Rabbits were exposed to atmospheres containing 2.3 (503 ppm), 1.06 (232 ppm), or 0.56 mg/L (121 ppm) of methylcyclohexanol (mixture of isomers), 6 hours daily, 5 days per week for 10 weeks. Mean daily urinary output of glucuronic acid conjugates during exposure is proportional to dose. Rabbits exposed to atmospheres containing 2.31 (514 ppm) or 0.816 mg/L (132 ppm) of methylcyclohexanone (mixture of isomers), 6 hours daily, 5 days per week for 10 weeks exhibit mean daily urinary output of glucuronic acid proportional to dose [Treon *et al.*, 1943b].

Rats received 500 mg/kg bw (128 microcurie/mg) of 3-^{3}H-2-isopropyl-5-methylcyclohexanol and urine and feces were collected 24 and 48 hours after dosing. The total excretion of 3-^{3}H-2-isopropyl-5-methylcyclohexanol by intact and bile duct-cannulated rats was greater than 70% of the dose at 48 hours. The glucuronic acid conjugate of 2-isopropyl-5-methylcyclohexanol and other minor oxidized metabolites are present in urine and fecal extracts. The glucuronic acid conjugate is also the main metabolite in the bile, while the glucuronic acid conjugate and minor metabolites (less than 5%) formed by side-chain oxidation are excreted in the urine [Yamaguchi *et al.*, 1994].

2.4.4 Summary of Metabolism

In summary, alkyl-substituted cyclohexanones are interconvertible with their corresponding alcohols *in vivo*. In the principal excretion pathway, the cyclohexanols are conjugated with glucuronic acid and excreted primarily in the urine.

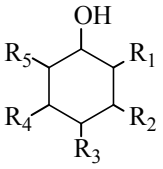
As indicated above, the major metabolic pathway involves reduction of the cyclohexyl ketones to yield the corresponding cyclohexanols that are subsequently excreted primarily as the glucuronic acid conjugates [Lington and Bevan, 1994; Topping *et al*., 1994; Cheo *et al.*, 1967; Elliott *et al*., 1965; Yamaguchi *et al.*, 1994]. To a very minor extent, alicyclic ketones and secondary alcohols containing an alkyl side-chain undergo oxidation of the side-chain to form polar poly-oxygenated metabolites that are also excreted as the glucuronide or sulfate conjugates mainly in the urine.

Although it has been anticipated that more lipophilic ketones or ketones with sterically hindered functional groups would undergo more extensive oxidation of alkyl ring substituents [Nelson *et al.*, 1992], studies with 2-, 3-, or 4-methylcyclohexanone, 2-isopropyl-5-methylcyclohexanol, 3,5,5-trimethylcyclohexanol, and even 2-, 3-, or 4-*tert*-butyl-substituted cyclohexanone or cyclohexanols reveal that conjugation of the cyclohexanol moiety by glucuronic acid is the predominant excretion pathway regardless of the size or position of the ring substituent. In general, the metabolic fate of alkyl-substituted cyclohexanone and cyclohexanol derivatives is similar to that of the un-substituted homologues (see Figure 2) [Lington and Bevan, 1994; Topping *et al*., 1994].

FIGURE 2 - METABOLIC FATE OF CYCLOHEXYL DERIVATIVES IN ANIMALS

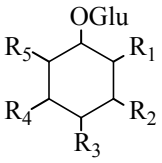
Unsubstituted or
Monosubstituted cyclohexanols
and cyclohexanones
R_1=H, CH_3-, $(CH_3)_3C$-
R_2=H, CH_3-, $(CH_3)_3C$-
R_3=H, CH_3-, $(CH_3)_3C$-

Disubstituted cyclohexanols
and cyclohexanones
R_1= $(CH_3)_2CH$-
R_4= CH_3



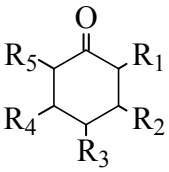
alkyl-substituted
cyclohexanol

major
route



glucuronic acid conjugate
of alkyl-substituted
cyclohexanol

Glu = glucuronic acid



minor
route

monogluconuride
of cyclohexanediol

Alkyl-substituted
cyclohexanone

In rats and rabbits, 66% of a 186 mg/kg bw dose of cyclohexanone or 47% of a 193 mg/kg bw dose of cyclopentanone *via* gavage is reduced to the corresponding secondary alcohol and excreted in the urine as the glucuronic acid conjugate [James and Waring, 1971]. Also, detected are trace amounts of mercapturic acid conjugate of the 2-hydroxycyclohexyl derivative [James and Waring, 1971]. Eighteen (18)-hour urine samples from rabbits administered 1,500 mg of cyclohexanone by gavage contain 65% cyclohexanol and a minor amount (6%) of *trans*-cyclohexane-1,2-diol as monoglucuronide conjugates [Elliott *et al.*, 1959]. Presumably, the diol forms by hydroxylation at the *alpha*-position of cyclohexanone followed by reduction of ketone function. The corresponding cyclohexanol derivative is the major urinary metabolite obtained from rabbits fed 460 mg/kg bw cyclohexane, 260 mg/kg bw cyclohexanol, or 350 mg/kg bw cyclohex-1-en-1-yl acetate [Elliott *et al.*, 1959].

The urine of rabbits given an oral dose of 1,200 mg/kg bw of cyclohexanol, shows a significant increase in glucuronic acid conjugates and decrease in inorganic sulfate compared to pre-dose levels [Treon *et al.*, 1943a]. The glucuronic acid conjugate of cyclohexanol is also obtained as the major urinary metabolite in rabbits given 890 mg/kg bw of cyclohexanone [Treon *et al*., 1943a]. The glucuronic acid conjugate of cyclohexanol (1.55 mg/L) and small amounts of cyclohexanone (0.23 mg/L) were found in the urine of workers occupationally exposed to a mixture of atmospheric hexanes including 456 mg/cu.m of cyclohexane [Governa *et al.*, 1987; Perbellini *et al.*, 1980]. The authors concluded that the cyclohexane is transformed to cyclohexanol that subsequently form glucuronic acid and sulfate conjugates.

Rats and rabbits were given oral doses of 200 - 3200 mg/kg bw of 2-, 3-, or 4-methylcyclohexanone. The glucuronic acid and sulfate conjugates of the corresponding secondary alcohols were the predominant urinary metabolites [Treon *et al.*, 1943a; Elliott *et al.*, 1959; Tao and Elliott, 1962].

Although the glucuronic acid conjugation of the alcohol is the predominant excretion pathway, oxidation of the alkyl substituents to yield poly-oxygenated metabolites has been reported as a minor pathway in animals. The number of possible polyoxygenated

metabolites increases with an increase in the types of alkyl ring substituents (*e.g.*, methyl and isopropyl substituents) [Nelson *et al*., 1992; Yamaguchi *et al*., 1994; Madyastha and Srivatsan, 1988; Asakawa *et al*., 1986].

The glucuronic acid conjugate of 2-, 3-, or 4-*tert*-butylcyclohexanol is the major urinary metabolite obtained 24 hours after rabbits were given 652 mg/kg bw of (±)-2-*tert*-butylcyclohexanone, 652 mg/kg bw of (±)-3-*tert*-butylcyclohexanone, or 562 mg/kg bw of 4-*tert*-butylcyclohexanone, respectively [Cheo *et al.*, 1967]. The mean percent of dose excreted is 76.5, 90, or 80% for 2-, 3-, or 4-*tert*-butylcyclohexanone, respectively. The ratio of *cis*- to *trans*-*tert*-butylcyclohexanol present in the urine of animals given 2-(71:29), 3-(74:26), or 4(26:74)-*tert*-butylcyclohexanone provides evidence that carbonyl reductase catalyzed reduction of the ketone function with NADH is influenced by steric effects of the *tert*-butyl substituent. The authors suggest that NADH uses a perpendicular approach to the carbonyl function in 2- and 3-*tert*-butylcyclohexanone. The 4-*tert*-butyl substituent, being more removed from the reaction cite, exerts only a minor impact on stereochemistry of the reduction of the ketone to the alcohol. In contrast, a "face to face" approach is used during the reduction of the corresponding smaller alkyl substituents (*e.g.*, methyl-substituted cyclohexanones) by NADH. In these cases, the *trans* isomer is favored [Elliott *et al.,* 1965].

The presence of multiple alkyl substituents at different positions on the cyclohexyl ring does not significantly alter the principal pathway of metabolism and excretion. 2-Isopropyl-5-methylcyclohexanone and the corresponding alcohol are mainly conjugated with glucuronic acid. At higher dose levels, *omega*-oxidation of the side chain substituents occurs to yield various polyols and hydroxyacids of 2-isopropyl-5-methylcyclohexanol [Yamaguchi *et al*., 1994; Madyastha and Srivatsan, 1988]. The unchanged alcohol and minor metabolites formed by side chain oxidation are eliminated in the urine and feces either unchanged or conjugated with glucuronic acid [Yamaguchi *et al*., 1994]. 2-Isopropyl-5-methylcyclohexanone is primarily reduced to the corresponding secondary alcohol that is then eliminated as noted above [Williams, 1940].

The metabolic fate of 2-isopropyl-5-methylcyclohexanol and 2-isopropyl-5-methylcyclohexanone has been studied in humans and other animals. Seventy-nine percent (79%) of a 1,000 mg oral dose [Quick, 1928] or 78% of a 10-20 mg oral dose [Atzl *et al.*, 1972] of 2-isopropyl-5-methylcyclohexanol administered to volunteers is eliminated as the glucuronic acid conjugate. For eight days, 750 mg of the *l* stereoisomer of 2-isopropyl-5-methylcyclohexanol was orally administered to three human volunteers followed by oral or intravenous administration of 200 mg [6-^{13}C]-glucuronolactone or [6-^{13}C]-sodium glucuronate. For two days after the administration of the isotopic compound, the glucuronic acid conjugate of 2-isopropyl-5-methylcyclohexanol is excreted in daily yields up to 84% of the 2-isopropyl-5-methylcyclohexanol administered [Eisenberg *et al*., 1955]. In two separate studies involving a total of 19 male and female volunteers, the glucuronic acid conjugate of 2-isopropyl-5-methylcyclohexanol is detected in the urine following oral administration of a 180 mg dose of an essential oil (peppermint oil) containing greater than 80% of 2-isopropyl-5-methylcyclohexanol, its stereoisomers, and the corresponding ketone [Kaffenberger and Doyle, 1990]. A 4,500 mg/kg bw oral dose of 2-isopropyl-5-methylcyclohexanol administered to rabbits is conjugated with glucuronic acid and eliminated in the urine [Deichmann and Thomas, 1943; Williams, 1939; Quick, 1924].

In rats, the vast majority of orally administered 2-isopropyl-5-methylcyclohexanol is eliminated in either the urine or feces as the glucuronic acid conjugate or, to a lesser extent, as various oxidation products of the alcohol [Yamaguchi *et al*., 1994; Madyastha and Srivatsan, 1988]. Non-cannulated and bile duct-cannulated male Fischer 344 rats (5/sex) were administered a single dose of 500 mg/kg bw of [3-^{3}H]-*l*-2-isopropyl-5-methylcyclohexanol. Urine and feces were collected over the next 24 and 48 hours in non-cannulated rats. In the bile duct-cannulated rats, bile samples were collected in two-hour intervals for the first six hours (3 collections) and then from 6-24 hours. Urine was collected at 24 hours.

In the non-cannulated rats, total recovery of the labeled substance in the urine or feces is 71.7% with the majority of the dose (45.4%) being recovered within the first 24 hours. In the urine, 37.8% percent of the radioactivity is excreted with equal amounts for the first

and second 24 hours. In the feces, 33.9% of the radioactivity is recovered with the majority in the first 24 hours (26.6%) [Yamaguchi *et al.*, 1994]. In the bile duct-cannulated rats, total recovery of the labeled substance in the urine or bile is 74.2% with the majority being recovered in the bile (66.9%). The bile metabolites are the mainly glucuronic acid conjugate of 2-isopropyl-5-methylcyclohexanol along with a variety of oxidation products in which the alkyl substituents (isopropyl or methyl substituents) of 2-isopropyl-5-methylcyclohexanol are oxidized [Yamaguchi *et al.*, 1994].

The biliary route of metabolism of 2-isopropyl-5-methylcyclohexanol and its corresponding ketone appear to be more important in rodents and dogs as compared to humans and rabbits. *l*-2-Isopropyl-5-methylcyclohexanone given to rabbits (1000 mg/kg bw) [Williams, 1940] is stereoselectively reduced to *d* stereoisomer of 2-isopropyl-5-methylcyclohexanol [Williams, 1940].

Urine samples collected over the course of four (4) days from rabbits given 1,000 mg/kg bw of isophorone (3,5,5-trimethyl-2-cyclohexen-1-one) *via* gavage showed several metabolites: the major conjugated metabolites includes 3,5,5-trimethyl-2-cyclohexen-1-ol (isophorol) formed by reduction of the ketone group and then conjugation with glucuronic acid and *cis*- and *trans*-3,5,5-trimethylcyclohexanol formed by hydrogenation of the endocyclic double bond, reduction of the ketone, and conjugation with glucuronic acid. In addition, 5,5-dimethyl-1-cyclohexene-3-one-1-carboxylic acid formed by methyl group oxidation at an exocyclic allylic position [Truhaut *et al*., 1970; Dutertre-Catella *et al.,* 1978].

The data clearly demonstrate that unsubstituted or alkyl-substituted cyclohexanones are readily absorbed and reduced to the corresponding cyclohexanol derivatives in a variety of animal species over a wide range of dose levels. The cyclohexanol derivatives are then conjugated with glucuronic acid and excretion mainly in the urine.

In summary, there is clear evidence that both the aliphatic linear- and branched-chain ketones and alkyl-substituted cyclohexanones in the KB4 and KB 3 mixtures are readily absorbed, interconverted with their corresponding alcohols, and excreted primarily as the glucuronic acid conjugate of the alcohol. Given the consistent pattern of

pharmacokinetics and metabolic fate, it is concluded that endpoint data for higher molecular weight linear aliphatic ketones and their corresponding alcohols and data on alkyl-substituted cyclohexanone and cyclohexanol derivatives are relevant to human hazard assessment of KB4 and KB3.

3 TEST PLAN

3.1 Chemical and Physical Properties

3.1.1 Melting Point

As expected, the increase in melting point for linear aliphatic ketones parallels and increase in molecular weight. The experimental melting points for 2-, 3-, and 5nonanone, 2-, 3-, and 4-decanone, 2-, 4-, and 6-undecanone, 2-, 3-, and 4-dodecanone, and 2-pentandecanone increase from -4.85 ºC to 39.5 ºC [CRC Handbook of Chemistry and Physics, 2000; Clayton and Clayton, 1994; Alarie *et al*, 1995; Perry and Green, 1984; MPBPVP EPI Suite, 2000, Syracuse Research Corporation]. The model prediction melting points for the same group of linear aliphatic ketones are in the range from –5.85 ºC to 46.2 ºC [MPBPVP EPI Suite, 2000] indicating good agreement between model predictions and actual experimental data. The experimental melting points of the branched chain aliphatic ketones 5-methyl-3-heptanone and 5-ethyl-2-nonanone are reported to be –56.7 and -7.03 ºC, respectively [Clayton and Clayton, 1994; MPBPVP EPI Suite, 2000].The calculated melting points for the 3,3-, 2,3-, 2,6-, and 2,4-dimethylcyclohexanone are in the range from –1.07 ºC to –13.73 ºC while that for 3-methyl-5-propylcyclohexanone is 9.03 ºC [MPBP VP EPI Suite, 2000].

Based on the above data, the melting points of chemically-identified aliphatic ketones of the KB4/KB3 mixture are in the range –5 ºC to 40 ºC. Lower experimental and model calculated melting point values for C_8 branched-chain aliphatic ketones and alkyl-substituted cyclohexanones are expected.

3.1.2 Boiling Point

The experimental boiling points for linear aliphatic ketones reflect both the influence of carbon chain length and the position of the ketone functional group. For 2-alkanones the boiling points are in the range from 192 ºC -195 ºC for 2-nonanone to 294 ºC for 2-

pentadecanone [CRC Handbook of Chemistry and Physics, 2000; MPBPVP EPI Suite, 2000; Fragrance Materials Association; Perry and Green, 1984]. For nonanone isomers, the boiling point of 2-nonanone is 192 ºC -195 ºC and that for 5-nonanone is 186 ºC 188 ºC [CRC Handbook of Chemistry and Physics, 2000; Clayton and Clayton, 1994], with the decrease reflecting the decrease in polarity expected as the ketone function moves to the middle of the aliphatic chain. Similar trends are observed for other alkanones. Model predicted values for the boiling points of the homologous series from nonanone to pentadecanone are in good general agreement with experimental values (*i.e.,* 184.65 ºC for nonanone to 291.95 ºC for pentadecanone) [MPBP VP EPI Suite, 2000]. However, the current model cannot account for the effect of the position of the functional group on the carbon chain. Therefore, the predicted values for the boiling points of 2-, 3-, 4-, or 5-nonanone are the same.

The reported boiling point of 6-methyl-2-heptanone is 171 ºC. The calculated boiling point of 212.54 ºC for 5-ethyl-2-nonanone is consistent with those of other undecanone isomers [MPBPVP EPI Suite, 2000]. The reported experimental boiling points for the 3,3-, 2,3-, 2,4-, and 2,6-dimethylcyclohexanones are in the narrow range from 171 ºC to 179 ºC [CRC Handbook of Chemistry and Physics, 2000] and the calculated boiling point of 3-methyl-5-propylcyclohexanone is 224.46 ºC [MPBPVP EPI Suite, 2000].

Given that the measured and calculated boiling points values for linear aliphatic ketones are consistent and reflect the influence of molecular weight and polarity, the boiling points of the homologous linear aliphatic ketones are expected to be in the range from 186 ºC to 294 ºC. The measured and calculated boiling points of the branched-chain ketones (6-methyl-2-heptanone and 5-ethyl-2-nonanone) and alkyl-substituted cyclohexanone derivatives identified in the KB4 /KB3 mixtures are in the range from 171 ºC to 225 ºC.

3.1.3 Vapor Pressure

The measured vapor pressure of 2-alkanones increases in the range from 0.642 mm Hg [Ohe, 1976] for 2-nonanone to 0.0216 mm Hg for 2-dodecanone [Perry and Green,

1984]. The vapor pressures of 2-, 3-, 4-, or 5-nonanone are similar. While 2-nonanone exhibits a vapor pressure of 0.642 mm Hg [Ohe, 1976], 5-nonanone shows a vapor pressure of 0.552 mm Hg [Alarie *et al.*, 1995]. Similar vapor pressures have also been observed for 2-undecanone (0.0414 mm Hg) [Perry and Green, 1984] and 6-undecanone (0.50 mm Hg) [Engineering Science Unit, 1975]. The calculated vapor pressures, for the homologous series from nonanone to pentadecanone are 0.99 to 0.0036 mm Hg [MPBP VP EPI Suite, 2000] and in good agreement with measured values.

The vapor pressures of the four dimethylcyclohexanone isomers are calculated to be in the range from 1.04 to 1.66 mm Hg [MPBP VP EPI Suite, 2000]. The calculated vapor pressure for 3-methyl-5-propylcyclohexanone is 0.158 mm Hg [MPBPVP EPI Suite, 2000]. Given the good agreement between measured and calculated vapor pressures, it is concluded that the vapor pressures of ketones in the KB4/KB3 mixtures are in the range of 0.0036 to 1.6 mm Hg.

3.1.4 n-Octanol/Water Partition Coefficients

Experimental log Kow values of 2-nonanone, 2-decanone, and 2-undecanone [Tanii *et al*., 1986] are 3.14, 3.73, and 4.09 respectively. The calculated log Kow values of 2.71, 3.21, and 3.69, respectively, for the same substances indicated that model values are similar to, but consistently less (approximately -0.4 units) than measured values [KOWWIN EPI Suite, 2000]. It should be emphasized that the calculated KOWWIN values are equivalent regardless of the position of the ketone. Values calculated based on a molecular fragment method [Hansch *et al.*, 1989] for 5-nonanone and 2-decanone (3.06 and 3.60, respectively) are in close agreement with experimental values.

The effect of the position of the ketone function on Kow values may be evaluated from experimental and calculated data for 2-nonanone and 5-nonanone. The experimental and calculated Kow values for 2-nonanone are 3.14 [Tanii *et al*., 1986] and 3.21 [KOWWIN EPI Suite, 2000], respectively. The experimental log Kow value for 5-nonanone is 2.88 [Abraham, 1994]. The Kow values calculated by the molecular fragment method for 5-nonanone are 2.79 [Hansch *et al*., 1967] and 3.06 [Hansch *et al.,* 1989]. The higher

values for 2-nonanone compared to those of 5-nonanone may indicate increased octanol solubility (higher log Kow) for the longer alkyl chain in 2-nonanone. However, it is difficult to evaluate these data given the different experimental and calculated methods used. In any event, the small difference between the experimental or calculated Kow values for 5-nonanone and 2-nonanone, indicate that the position of the ketone function has little impact on the log Kow.

Based on the results of log Kow determinations for a series of ketones, including 7-tridecanone (log Kow = 5.17) using a molecular fragment method [Hansch and Leo, 1979], it is estimated that log Kow of 2-dodecanone is 4.60. Similar to experimental and calculated values for other lower molecular weight ketones, the KOWWIN calculated log Kow of 4.18 [KOWWIN EPI Suite, 2000] is slightly lower than values calculated by the molecular fragment method. Based on the above analysis, the log Kow values for the isomers of nonanone, decanone, undecanone, and dodecanone are concluded to be 3.17, 3.73, 4.09, and 4.60, respectively. The calculated log Kow of 5.66 [KOWWIN EPI Suite, 2000] for 2-pentadecanone is consistent with the trend of experimental and calculated values for lower homologues.

The calculated value of log Kow for 6-methyl-2-heptanone is calculated to be 2.15 [KOWWIN EPI Suite, 2000]. The log Kow values calculated by a molecular fragment method for the linear isomer, 2-octanone, are 2.52 [Hansch *et al.*, 1989] and 2.46 [Hansch and Leo, 1979]. Given that alkyl branching increases water solubility, the difference recorded for the straight and branched chain isomers is reasonable. Therefore, the log Kow for 6-methyl-2-heptanone is concluded to be 2.15. Comparison of calculated log Kow values for straight (2-undecanone, log Kow 3.69) and branched-chain (5-ethyl-2-nonanone, log Kow 2.94) ketones reveals a similar trend. The dimethylcyclohexanone derivatives are calculated [KOWWIN EPI Suite, 2000] to exhibit log Kow value of 1.98 while 3-methyl-5-propylcyclohexanone exhibits a calculated log Kow value of 2.94. Based on the above analysis, the ketones in KB4/KB3 exhibit log Kow values in the range of 2-4. Only pentadecanone isomers in KB3, that account for only 5% of the mixture, exhibit a log Kow value greater than 5.

3.1.5 Water Solubility

The experimental water solubility of 376 mg/L determined for 5-nonanone [Palit, 1947] is in good agreement with the calculated value of 284.4 mg/L [WSKOWWIN EPI Suite, 2000]. Other calculated values for 2-, 3-, 4-, and 5-nonanone are in the range from 170.6 to 396.1 mg/L [WSKOWWIN EPI Suite, 2000]. The water solubilities for the isomers of decanone is 46.4 to 131 mg/L, for undecanone is 19.7 to 43.0 mg/L, for dodecanone is 14.0 mg/L, and for pentadencanone is 0.468 mg/L. The calculated water solubilities of 6-methyl-2-heptanone and 5-ethyl-2-nonanone are determined to be 1,371 mg/L and 49.63, respectively, at 25 $^{\circ}$C [WSKOWWIN EPI Suite, 2000]. The calculated water solubilities of the isomers of dimethylcyclohexanone and 3-methyl-5-propylcyclohexanone are determined to be 1,874 mg/L and 222.7, respectively, at 25 $^{\circ}$C [WSKOWWIN EPI Suite, 2000]. With the exception of pentadecanone, the solubility range for aliphatic and alicyclic ketones in the KB4/KB3 mixtures is expected to be approximately 50-2000 mg/L.

3.1.6 New Testing Required

No further testing is required.

3.2 Environmental Fate and Pathways

3.2.1 Photodegradation

The measured half-life values for the reaction of 2-octanone, 2-nonanone, and 2decanone with hydroxyl radicals based on an average atmospheric hydroxyl radical concentration of 5 x10^{5} molecules/cm^{3} have been reported to be 5.5, 6.3, and 6.8 hours, respectively [Wallington and Kurylo, 1987]. Calculated half-lives for the 2-nonanone and 2-decanone are 11.67 and 10.34 hours, respectively [AOPWIN EPI Suite, 2000]. The calculations are based on measured rate constants for radical reactions of OH with organic substrates [AOPWIN EPI Suite, 2000]. The short half-lives for the ketones are consistent with the presence of reactive *alpha*- and *beta*-hydrogens (hydroxyl radical-*beta* hydrogen 6-membered trans ition state) present in the aliphatic ketones. The calculated and measured values for rate constants for aliphatic ketones are consistently in the range of 10 X 10^{-12} to 10 X 10^{-13} cm^{3}/molecule-second while the half-lives are in the range from

5.5 to 12 hours. Also, calculated half-lives for the alkyl-substituted cyclohexanone derivatives and branched-chain ketones are in the range of 4.8 to 16 hours [AOPWIN EPI Suite, 2000]. Taken together these data support the conclusion that the ketones in KB4/KB3 mixtures will be rapidly degrade in the atmosphere atmosphere.

3.2.2 Stability in Water

Hydrolysis is the reaction of organic molecules with water under acidic conditions to yield products in which new bonds to oxygen (OH) and hydrogen (H) are formed such as in the hydrolysis of esters to yield a carboxylic acid and an alcohol. Hydrolysis may also involve the addition of water to aldehydes or ketones to yield acetals or ketals under mild acid conditions. However, this addition of water is thermodynamically favorable only for low molecular weight aldehydes (*e.g.*, acetaldehyde) or ketones. The higher molecular weight ketones in KB4/KB3 do no form stable ketals. Therefore, the ketones are stable to water under ambient environmental conditions.

Another possible reaction of ketones in water involves the enolic hydrogen on the carbons bonded to the carbonyl function. Under conditions of high pH (pH greater than 10), the enolic proton is abstracted by base (OH^-) forming a carbanion intermediate that may react with other organic substrates (*e.g.,* ketones, esters, aldehydes) containing a center for nucleophic attack. The reactions, commonly recognized as condensation reactions, produce higher molecular weight products. Under ambient conditions of temperature, pH, and low concentration, these condensation reactions are unfavorable. Therefore, under environmental conditions, it is concluded that the ketones in the KB4/KB3 mixtures are stable.

3.2.3 Biodegradation

In a study adhering to OECD Guidelines, 5-methyl-2-hexanone was readily biodegradable (67% in 14 days) when tested using activated sewage sludge sewage [Springborn Laboratories, 2001]. 2-Heptanone was reported to be biodegradable when tested with domestic activated sludge in the ISO BOD [Eastman Chemical Co., 1997b] or COD test [Eastman Che mical Co., 1997a]. The C11 ketone, 2,6,8-trimethylnonanone, was not readily biodegrable (44.7% degraded after 28 days) according to an OECD 301D guideline study (Dow Chemical, 2004)

Based on the BIOWIN Model, alkanones from C9 to C15 are determined to be readily biodegradable by the Linear or Non-linear Biodegradation Probability Models [BIOWIN EPI Suite, 2000]. Given the the experimental data on C-7 to C-11 ketones, it can be conclude that higher molecular weight ketone (>C-11) will biodegrade but only after 28 days.

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model [Mackay, 1991, 1996a, 1996b]

through the EPA EPI Suite 2000 program. The input parameters used were molecular weight, melting point and boiling point.

The model predicts that linear aliphatic ketones are distributed mainly to the soil (48-67%), but are also distributed to water (16-36%) with the lower molecular weight ketones (nonanones) showing slightly greater distribution to the air (4-6%) than the higher molecular weight ketones (pentadecanones) (2%). Based on structural features of these substances, distribut ion to air and sediment (0.7-3.0%) are expected to be minimal for the major linear aliphatic ketones in the KB4/KB3 mixture. Half-lives in the environment are calculated to be less than a day in the air (13-30 hours), weeks (2-10) in water and soil, and months (1-3) in the sediment.

Model caluclations for the isomeric dimethylcyclohexanones, 3-methyl-5-propylcyclohexanone, 5-ethyl-2-nonanone, and 6-methyl-2-heptanone predict environmental fates similar to the linear aliphatic ketones discussed above. Based on the ready biodegradability data and the predicted half-lives in the various environmental compartments, it is concluded that the majority of ketones in KB4/KB3 mixtures are not persistent in the environment.

3.2.5 New Testing Required

No further testing is required.

3.3 Ecotoxicity

3.3.1 Acute Toxicity to Fish

Experimental and calculated acute toxicity data for fish are available for 2-octanone [Veith *et al*., 1983; Broderius and Kahl, 1985], 2-nonanone [Geiger *et al.*, 1986], 5-nonanone [Veith *et al*., 1983; Geiger *et al.*, 1986], 2-decanone [Geiger *et al.*, 1986], 2-undecanone [Geiger *et al.*, 1986] and 2-dodecanone [Geiger *et al.*, 1986] (see Table 2). When tested at its limit of solubility, 2,6,8-trimethyl-4-nonanone show no toxic effects to Oncorhynchus mykiss in an OECD 203 guideline study (Dow Chemical, 2004). These values compared favorably with the LC50 values calculated for ketones as neutral organics in the ECOSAR model [ECOSAR EPI Suite, 2000].

TABLE 2 - ACUTE 96-HR LC50 VALUES IN FATHEAD MINNOW (FLOW-THROUGH METHOD)

Substance	Calculated 96-hour LC50, mg/L	Experimental 96-hour LC50, mg/L	Reference
2-Octanone	63.0	NA	Broderius and Kahl, 1985
2-Nonanone	22.68	15.2	Geiger *et al.*, 1986
5-Nonanone	22.68	31	Veith *et al*., 1983; Geiger *et al.*, 1986
2-Decanone	8.627	5.7	Veith *et al*., 1983; Geiger *et al.*, 1986
2-Undecanone	3.255	1.5	Geiger *et al.*, 1986
2-Dodecanone	1.20	1.18	Geiger *et al.*, 1986

2-Pentadecanone	0.06	NA	NA

Based on these values, it is concluded that the range of 96-hour LC50 values for the homologous series from 2-nonanone to 2-dodecanone is 31 to 1.18 mg/L. Based on the ECOSAR model it is anticipated that the minor amount (5%) of pentadecanone isomers in KB3 will exhibit the lowest LC50 (approximately 0.06 mg/L) [ECOSAR EPI Suite, 2000].

Acute 96-hour LC50 data on isomers and substances structurally related to 6-methyl-2-heptanone indicate an LC50 value between 50 and 100 mg/L for. In tests using a continuous flow-through me thod, 2-octanone and 6-methyl-5-hepten-2-one exhibit 96-hour LC50 values of 36mg/L and 85.7 mg/L, respectively [Veith *et al.,* 1983]. In a second flow through study, 2-octanone was reported to show a 96-hour LC50 value of 63 mg/L [Broderius and Kahl, 1985]. Also, the homologue, 5-methyl-2-hexanone exhibited a 96-hour LC50 value of 100 ul/L (80.2 mg/L) in a static test using fathead minnows [Eastman Kodak Co., 1978]. These values are in good agreement with the 96-hour LC50 value of 68.70 calculated for 6-methyl-2-heptanone by the ECOSAR model [ECOSAR EPI Suite, 2000]. For the other branched-chain isomer of undecanone, 5-ethyl-2-nonanone the calculated 96-hour LC50 is 3.788 mg/L [ECOSAR EPI Suite, 2000]. The experimental 96-hour LC50 of 3,5,5-trimethylcyclohexenone (220 mg/L) in bluegills determined under static conditions [Buccafusco *et al.*, 1981] compares favorably with the calculated values of 93.55 mg/L for 3,3-dimethylcyclhexanone and 102 mg/L for 2,3-, 2,4-, or 2,6-dimethylcyclohexanone determined by the ECOSAR model [ECOSAR EPI Suite, 2000]. The calculated 96-hour LC50 value of 14.95 mg/L for the more lipophilic cyclohexanone, 3-methyl-5-propylcyclohexanone, is consistent with other model predictions for more lipophilic alkyl-substituted cyclohexanones [ECOSAR EPI Suite, 2000].

Given the consistency of measured and calculated data for representative alkanones in the KB4/KB3 mixtures, it will not be necessary to perform additional acute fish toxicity tests.

3.3.2 Acute Toxicity to Invertebrates

Calculated aquatic invertebrate 48-hour LC50 values for nonanone, decanone, undecanone, dodecanone, and pentadecanone decrease in the order of 26.52, 10.04, 3.92, 1.52, to 0.084 mg/L [ECOSAR EPI Suite, 2000]. These values are consistent with, and similar to, the respective acute toxicity values calculated for fish [ECOSAR EPI Suite, 2000]. Also, the calculated 48-hour LC50 values for 6-methyl-2-heptanone (74.38 mg/L), 5-ethyl-2-nonanone (4.54 mg/L), 3,3-dimethylcyclohexanone (93.88 mg/L), and 2,3-, 2,4-, or 2,6-dimethylcyclohexanone (109.0 mg/L) are remarkably similar to the calculated 96-hour LC50 values for fish.

The experimental 96-hour LC50 value and 96-hour EC50 value of the homologue 5-methyl-2-hexanone in *Daphnia magna* are reported to be greater than 100 ul/L (greater than 80 mg/L) [Eastman Kodak Co., 2000]. This value is consistent with the ECOSAR calculated value of 68.70 mg/L for 6-methyl-2-heptanone and with the experimental 24-hour LC50 value (170mg/L) for 5-methyl-2-hexanone in *Daphnia magna* [Bringmann and Kuehn, 1977]. Other linear (3-octanone) and cyclic (2-methylcyclohexanone) ketones exhibit 24-hour LC50 values (517 and 435 mg/L, respectively) that indicate a low order of acute toxicity for the component ketones in the KB4 /KB3 mixtures [Bringmann and Kuehn, 1977].

In 48-hour test using *Tetrahymena pyriformis*, the EC50 values for 4-heptanone, 2-octanone, 5-nonanone, and 2-decanone were reported to be 679, 224, 145, and 49.3 mg/L, respectively [Schultz *et al.*, 1990]. In a 40-hour static test, again using *Tetrahymena pyriformis*, the 50% growth inhibitory concentrations of 2-nonanone, 2-decanone, 2-undecanone, and 2-dodecanone are reported to be 33.26, 44.21, 5.76, and 4.19 mg/L, respectively [Schultz, 1997]. Given that these EC50 values were measured over a shorted time period, lower EC50 values are anticipated. This is exemplified by a recent study on a C-11 ketone. In a separated OECD 202 Guideline study, the EC50 value for 2,6,8-trimethyl-4-nonanone is 3.41 mg/L (Dow Chemical, 2003). Based on this value, EC50 values of ketones containing less than 15 carbons is expected to be >1 mg/L.

Based on the experimental and calculated values for ketones in the KB4/KB3 mixture, with the exception of pentadecanone isomers, no ketone is predicted to exhibit a 96-hour LC50 value less than 1.0 mg/L.

3.3.3 Acute Toxicity to Aquatic Plants

Experimental acute toxicity data for aquatic plants were available for 2-heptanone and 5-methyl-2-hexanone. 2-Heptanone [Eastman Kodak Co., 1998] and 5-methyl-2-hexanone [Eastman Kodak Co., 2001a] were tested in *Scenedesmus subspicatus* (algae) and a 72-hour EC50 value of 42.7 mg/L was determined in both tests. The experimental values are on the same order of magnitude as the ECOSAR calculated value of 98.3 mg/L for 2-heptanone [ECOSAR EPI Suite 2000]. In an OECD 201 guideline study, 2,6,8-trimethyl-4-nonanone showed no toxicity to *Pseudokirchneriella subcapitata* at a concentration of 1.03 mg/L, the limit of solubility (Dow Chemical, 2003). Therefore, the EC50 value exceeds the 1.0 mg/L level.

Model calculated 96-hour EC50 values for all isomers of nonanone, decanone, undecanone, dodecanone, and pentadecanone decrease in the order 16.62, 6.728, 2.701, 1.077, and 0.065 mg/L, respectively. Also, calculated 96-hour EC50 values for isomers of dimethylcyclohexanone and 6-methyl-2-heptanone are 62.67 and 46.90 mg/L, respectively [ECOSAR EPI Suite, 2000]. Based on the consistency of the measured and calculated values for 2-heptanone and 5-methyl-2-hexanone, the ECOSAR calculated values for higher homologues are reliable. They indicate that in the homologous series from C_7-C_{12} the 96-hour EC50 values are in the range from 100 to greater than 1 mg/L.

3.3.4 New Testing Required

No further testing is required.

3.4 Human Health

Based on the consistent pattern of pharmacokinetics and metabolic fate that indicates ketones and alcohols are interconvertible *in vivo*, it is concluded that endpoint data for higher molecular weight linear aliphatic ketones and their corresponding alcohols and data on alkyl-substituted cyclohexanone and cyclohexanol derivatives are relevant to human hazard assessment of KB4/KB3 mixtures.

3.4.1 Acute Toxicity

Given the relatively low volatility of higher molecular weight ketones, acute toxicity *via* the oral and dermal routes would be more significant than *via* the inhalation route of exposure. Numerous oral, dermal, and inhalation LC50 values for linear aliphatic and alicyclic ketones have been reported in rats, mice, and rabbits. Overall, they exhibit a low acute toxic potential. Oral and dermal LC50s tended to exceed 5,000 mg/kg, with some oral LD50 values approaching 20,000 mg/kg.

For 2-nonanone, the rat oral LC50 was reported to be greater than 5,000 mg/kg bw [Moreno, 1980] in rats and 7,879 mg/kg [Tanii *et al*, 1986] in ddY mice. The oral LD50 value of 3-nonanone (LD50 = 5270 mg/kg) [Hoffman-Laroche, 1967] is similar to that of 2-nonanone. For 2-decanone, the rat oral LC50 value was reported to be 7,940 mg/kg [Tanii *et al*., 1986]. The oral and dermal acute LD50 values of 2-undecanone are reported to exceed 5,000 mg/kg [Levenstein, 1974]. This oral LD50 value is consistent with another oral LD50 value of 19,448 mg/kg [Tanii *et al*., 1986] reported for 2-undecanone. The oral LD50 value for 2-tridecanone is also reported to be greater than 2,000 mg/kg [Dragoco, 2000].

Oral and dermal LC50 values for branched chain ketones are in the same range as for linear aliphatic ketones. The oral and dermal LD50 of 6-methyl-5-hepten-2-one is reported to be 4,100 mg/kg and greater than 5,000 mg/kg, respectively [Keating, 1972]. In another study on the same substance the oral LD50 was reported to be 4,200 ul/kg while, in an inhalation study, no deaths were recorded in rats exposed for 8 hours to an

atmosphere saturated with 6-methylheptenone at 20 ^{o}C [BASF, 1974]. The oral and inhalation LD50 of 3,5,5-trimethylcyclohexenone in Wistar rats is reported to be 3,450 mg/kg and 7 mg/L (1291 ppm), respectively [Exxon Chemical Americas, 1982].

Given the current database of information, it is concluded that the aliphatic and alicyclic ketones in the KB4/KB3 mixtures are of low acute oral or dermal toxicity. It will not be necessary to perform additional acute toxicity tests.

3.4.2 *In vitro* and *In vivo* Genotoxicity

Representative aliphatic and alicyclic ketones have been tested in *in vitro* bacterial and mammalian cell lines and have shown no mutagenic or genotoxic potential. Similar results have been reported for the ketones and corresponding alcohols in *in vivo* assays. These findings confirm that the aliphatic ketones and alkyl-substituted cyclohexanones in the KB4/KB3 mixtures exhibit a low genotoxic potential.

3.4.2.1 In vitro Genotoxicity

Ames assays were performed on four aliphatic ketones of chain length greater than C_8. 2,6-Dimethyl-4-heptanone [Mortelmans *et al*., 1986], 6-methyl-5-hepten-2-one [Florin *et al*., 1980], and 6,10-dimethyl-2-undecatrienone [Florin *et al*., 1980] show no evidence of mutagenicity in TA98, TA100, TA1535, TA1537 and TA1538 strains of *Salmonella typhimurium*.

In vitro mutagenicity Ames testing has been performed with three alkyl-substituted alicyclic ketones. Negative results were reported in the standard Ames assay when various strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) were incubated with 3,5,5-trimethylcyclohexenone [Mortelmans *et al.*, 1986], 2,2,6-trimethylcyclohexanone [Florin *et al.*, 1980] (in TA 98 and TA100 only), or tetramethylethylcyclohexanone [Wild *et al.*, 1983] with or without S-9 metabolic activation.

3.4.2.2 *In Vivo Genotoxicity*

When 2-hexylidenecyclopentanone, tetramethylethylcyclohexanone, or 3,5,5-trimethylcyclohexenone were fed to adult *Drosophila melanogaster* for 3 days no mutations were observed [Foureman *et al*., 1994; Wild *et al.*, 1983]. In addition, negative results were obtained when *Drosophila melanogaster* were injected with a single dose of 12,500 micrograms 3,5,5-cyclohexenone [Foureman *et al.*, 1994].

There was no increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of male or female CD-1 mice administered 498 mg/kg bw of 3,5,5-cyclohexenone by intraperitoneal injection [O'Donoghue *et al*., 1988] or in NMRI mice intraperitoneally injected with 166, 333 or 500 mg/kg bw of 2-hexylidenecyclopentanone or 180, 307 or 450 mg/kg bw of tetramethylethylcyclopentenone [Wild *et al.,* 1983].

Intraperitoneal injection on 3 consecutive days with up to 1,000 mg/kg bw of 2-isopropyl-5-methylcyclohexanol did not induce micronuclei in mouse bone marrow [Shelby *et al*., 1993]. In a host-mediated assay, mice were gavaged with up to 3,000 mg/kg bw of 2-isopropyl-5-methylcyclohexanol in a single-dose study or up to 1,150 mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol in a 5-day study [Food and Drug Administration, 1975]. After the last dose, mice were intraperitoneally injected with an indicator organism (*Salmonella typhimurium* strains G46 and TA1530, or *Saccharomyces cervisiae* D3). The peritoneal exudate was plated and incubated for assessment of mutation and recombinant frequencies. No significant increase in mutant and recombinant frequency at any dose or exposure period in *Salmonella typhimurium* G46. In *Saccharomyces cervisiae* D3, an elevation of recombinant frequency was reported in the 5-day exposure study, but not in the single-exposure study. At the highest dose tested in *Salmonella typhimurium* TA1530, in the single-dose study, a significant increase in mutant frequency was reported. This was not reported in the 5-day study. *In vitro* tests using the same organisms were all negative.

In a chromosomal aberration study, rats were gavaged with up to 3,000 mg/kg bw of 2-isopropyl-5-methylcyclohexanol as a single exposure or up to 1,150 mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol for 5 days [Food and Drug Administration, 1975].

Analysis of bone marrow demonstrated that exposure to 2-isopropyl-5-methylcyclohexanol as a single dose or for a 5-day period did not induce chromosomal aberrations.

3.4.2.3 Conclusions

The *in vitro* studies, on linear and branched-chain aliphatic ketones and alicyclic ketones, show no evidence of genotoxicity. The *in vivo* studies, performed on alkyl-substituted alicyclic ketones and structurally related alcohols, also show no evidence of genotoxicity. Therefore, it is concluded that, the mixture of ketones in KB4/KB3 mixtures is of low genotoxic potential.

3.4.3 Repeat Dose Toxicity

Repeat-dose toxicity studies are available for 2-nonanone, 2,6-dimethyl-4-heptanone, 5-nonanone, an undecanone isomer, 2,8-dimethyl-5-nonanone, 3,5,5-trimethyl cyclohexenone, a mixture of alkyl-substitued cyclohexanones and cyclohexanols, and the alkyl-substituted cyclohexanol derivative, 2-isopropyl-5-methylcyclohexanol. The most consistent pathologic effect in both subchronic and chronic studies was the appearance of hyaline droplet nephropathy in male rats.

Groups of 3 Charles River CD, COBS male rats were administered 2-nonanone (*i.e.,* methyl heptyl ketone) *via* gavage, 5 days per week for 3 weeks at doses of 1,000, 2,000, or 4,000 mg/kg bw. Individual body weights and feed consumption were recorded on days 0, 3, 7, 14 and 20 of treatment. All animals were observed daily for clinical signs of toxicity. Necropsy was performed on all test animals, and tissues were collected for histological examination.

Upon necropsy, no gross compound-related changes were detected at any dose level. Histological examination revealed compound related changes in the stomach and liver at the 2,000 and 4,000 mg/kg bw/day levels, and in the lungs, kidneys, bladder, adrenal glands, bone marrow, brain, and mesenteric fat at the 4,000 mg/kg bw/day level. However, it was not reported whether or not these effects were statistically significant.

In the stomach, hyperplasia of the epithelium of the non-glandular mucosa was observed with at varying degrees, which were thought to reflect the amount of contact the test material had with the epithelium and the selection of the tissue specimens for examination. Liver changes were characterized by hepatocyte hypertrophy. In the 4,000 mg/kg bw/day group, lungs showed minor acute bronchitis and congestion, edema, and atelectasis; the urinary system had dilatation of the lumena of the renal tubules and multiple hemorrrhages in the bladder (1 rat); the adrenal gland was congested; bone marrow and brains were congested in 2 or 3 rats, respectively; and atrophy of the mesenteric adipose tissue occurred in 1 rat. In the 1,000 mg/kg test group, no gross or histopathologic compound-related changes were identified [Krasavage and O'Donoghue, 1979].

2,6-Dimethyl-4-heptanone (67.0% purity; *i.e.*, diisobutyl ketone) was administered to 8 male Charles River rats by gavage for 90 days at a dose of 0 or 2,000 mg/kg bw/day. Following the dosing period, liver, kidney, brain, adrenal glands, testes, heart and spleen weights were recorded and relative organ weights calculated. Hematology and clinical chemistry was performed and results were comparable to controls. Absolute and relative liver weights, relative kidney weights, and absolute and relative adrenal gland weights were statistically greater than controls. Absolute, but not relative brain and heart weights were significantly depressed. All other organ weights were comparable to controls.

No compound related gross pathologic changes were identified. Histopathology examinations were also conducted on the test animals and revealed compound related changes in the stomach, liver, and kidneys. In the stomach, all animals showed hyperkeratosis or hyperkeratosis with pseudoepitheliomatous hyperplasia associated with irritation from direct contact by the solvent. In the liver, minor or moderate hepatocyte hypertrophy was observed. In the kidney, hyaline droplet formation was present in the proximal tubular epithelium suggesting *alpha*-microglobulin-type nephropathy. There was also a minor occurrence of regenerating tubular epithelium and tubular dilation with casts [O'Donoghue and Krasavage, 1980]. A similar toxicologic profile including the presence of hyaline droplet formation was reported after 2,000 mg/kg of 5-methyl-2-hexanone [Eastman Kodak Co., 1979] or 4000 mg/kg of 2,8-dimethyl-5-nonanone

(99.5%) [O'Donoghue and Krasavage, 1980] was given to rats 5 days per week for 90 days.

In a study limited to the neurotoxic evaluation, groups of Charles River male rats were given 233 mg/kg of 5-nonanone (98.25%) 5 days weekly for 90 days. There was no observable evidence of neurotoxic activity. However, at the 233 mg/kg bw dose level slight neuropathologic changes were observed (myelin ovoid formation, remyelination or adaxonal myelin in 3 of 5 rats [O'Donohogue J. L. *et al.*, 1982]. In an oral gavage study, 2,000 mg/kg of impure 2-nonanone (11% 5-nonanone, approximately equal to 233 mg/kg bw) when administered to rats for 90-days showed similar evidence of "giant axonal swelling" neuropathy. This neurotoxic phenomenom is typical of ketones that can be readily metabolized to *gamma* diketones (*i.e.*, 5-nonanone) [O'Donoghue and Krasavage, 1980]. However, when fed to rats at lower dietary levels (<200 mg/kg bw), 5-nonanone showed no evidence of neurotoxicity.

In a 14 day dietary study, 2-pentadecanone was added to the diet of groups of male and female Fischer 344 rats at levels calculated to provide an average daily intake of 10 mg/kg bw. Based on measurement of body weight, food consumption, gross examination, liver and kidney weights, and microscopic examination of the liver and kidneys, no effects were observed [VanMiller and Gill, 1987].

A sample of an essential oil, predominantly containing a mixture of 2-isopropyl-5-methylcyclohexanone and 2-isopropyl-5-methylcyclohexanol isomers that accounts for greater than 85% of the mass of the oil, was used in the 28 day study [Serota, 1990] and reproductive/developmental screening [Hoberman, 1989, robust summary in reproductive toxicity section] study cited below. Based on a gas chromatogram (FIS detector), the oil was determined to contain:

- 46.8% (1 *alpha*, 2 *beta*, 5 *alpha*)-2-isopropyl-5-methylcyclohexanol
- 3.97% (1 *alpha*, 2 *alpha*, 5 *alpha*)-2-isopropyl-5-methylcyclohexanol

- 0.86% (1 *beta*, 2 *beta*, 5 *alpha*)-2-isopropyl-5-methylcyclohexanol
- 21.81% (2 *beta*, 5 *alpha*)-2-isopropyl-5-methylcyclohexanone
- 3.07% (2 *beta*, 5 *beta*)-2-isopropyl-5-methylcyclohexanone
- 5.11% (1 *alpha*, 2 *beta*, 5 *alpha*)-2-isopropyl-5-methylcyclohexyl acetate
- 1.55% (1 *beta*, 2 *beta*, 5 *beta*)-2-isopropyl-5-methylcyclohexyl acetate

The other constituents accounting for approximately 10% of the oil included aliphatic terpene hydrocarbons (*e.g.*, *alpha*-pinene) and ethers (eucalyptol) [Vollmuth, 1989, no robust summary provided].

The sample was administered by gavage in corn oil to groups of Sprague-Dawley rats at dose levels of 0, 100, 200, or 400 mg/kg bw/day for 29 or 30 days [Serota, 1990]. Clinical signs, body weights and food consumption were monitored. At necropsy, organ weights (brain, spleen, liver, heart, kidneys, testes with epididymides, adrenals, ovaries, and pituitary) were measured, and tissues (26) were preserved in 10% formalin. All tissues from the control and high-dose groups and tissues from the heart, liver, kidneys, and gross lesions from the low- and mid-dose group were embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically. All animals survived to study termination with high-dose males showing increased incidence of urine staining during clinical observations. Except for a non-statistically significant decrease in mean body weight in high-dose males, there were no statistically significant differences in body weight or food consumption between treated and control groups. A significant decrease in serum glucose levels was reported in the mid- and high-dose males that the authors, in part, attribute to change in nutritional status as revealed by a decreased body weights in the high-dose group. A treatment-related increase in alkaline phosphatase also was reported in high-dose males. Measurement of body weight, food consumption, hematology and clinical chemistry parameters revealed no significant changes between test and control female rats. There were statistically significant increases in relative kidney weights in high-dose males. Histopathological findings revealed renal tubule protein droplets in all groups of treated male rats. The authors considered these findings related to the lysosomal handling of *alpha*-2-microglobulin, a protein specific to the male Sprague-Dawley rat. Absolute and relative liver weights in high-dose females also were

significantly increased but these changes were not confirmed by histopathological examination. There was no histopathology of tissues from reproductive organs of males (testes with epididymis) or female (ovaries). Based exclusively on the renal pathology reported in all dosed groups of male rats, the authors concluded that the no observable adverse effect level (NOAEL) for the sample is less than 100 mg/kg bw/day in male rats and 400 mg/kg bw/day in female rats.

The National Toxicology Program (NTP) conducted a chronic two-year bioassay on 3,5,5-trimethylcycloheneone (isophorone) using the standardized NTP protocol in F344/N rats. Doses were determined from the results of a prior 13-week subchronic toxicity study.

In a two-year study dose levels of 0, 250 or 500 mg/kg bw/day of 3,5,5-trimethylcycloheneone were given to groups of F344/N rats (50/sex/group) by gavage in corn oil 5 days a week daily for 103 weeks [NTP, 1986; Bucher *et al.*, 1986]. Food and water were provided *ad libitum*. Moribund animals were euthanized. Weights were recorded weekly and at the termination of the experiment survivors were sacrificed and necropsies performed. No clinical signs of toxicity were reported. Gavage errors accounted for a significant number of deaths (36/300) in both male and female rats.

Nephropathy was noted in both test and control rats of both sexes after natural death or at termination. In test animals, increased incidence of mineral deposits in renal collecting ducts (31/50, 62% and 20/50, 40%), and tubular cell hyperplasia (1/50, 2% and 4/50, 8%), adenomas (0/50 and 2/50, 8%), and adenocarcinomas (3/50, 6% and 1/50, 2%) were observed in male rats at 250 mg/kg bw/day and 500 mg/kg bw/day, respectively but not in female rats (see Table 3). Tubule mineralization was characterized by basophilic aggregates found in the medullary collecting ducts, often occurring coincidentally with lesions of chronic nephropathy. Authors of the NTP report concluded the following: "Under conditions of these 2-year gavage studies, there is some evidence of carcinogenicity of 3,5,5-trimethylcycloheneone in the male F344/N rat as shown by the occurrence of renal tubular cell adenomas and adenocarcinomas in animals given 250 or 500 mg/kg/d..." [NTP, 1986; Bucher *et al*., 1986].

TABLE 3 - INCIDENCES OF RENAL NEOPLASMS ASSOCIATED WITH ADMINISTRATION OF 3,5,5-TRIMETHYLCYCLOHEXENONE TO RATS BY GAVAGE FOR 103 WEEKS

		Control	250 mg/kg	500 mg/kg
1.	Male Rats			
	Nephropathy	49/50	47/50	46/50
	Tubule mineralization	1/50	31/50	20/50
	Renal Tubule Hyperplasia	0/50	1/50	4/50
	Renal Tubule Adenoma*	0/50	0/50	2/50
	Renal Tubule Adenocarcinoma*	0/50	3/50	1/50
2.	Female Rats			
	Nephropathy	21/50	39/50	32/50
	Tubule mineralization	10/50	4/50	2/50
	Renal Tubule Hyperplasia	0/50	0/50	1/50
	Renal Tubule Adenoma*	0/50	0/50	0/50
	Renal Tubule Adenocarcinoma*	0/50	0/50	0/50

* Historical incidence of tubular cell adenoma or adenocarcinoma: 4/1091 (0.4%) $p < 0.05$.

B6C3F1 mice were fed diets containing 0, 930, 1870, 3750, 7500, or 15,000 ppm *dl*-2-isopropyl-5-methylcyclohexanol (approximately 0, 140, 281, 563, 1125 or 2,250 mg/kg bw/day of *dl*-2-isopropyl-5-methylcyclohexanol, respectively) for 13 weeks [National Cancer Institute, 1979]. Necropsies were performed on all animals at the end of the study. Histopathological examination was performed on tissues from selected animals. Six mice (sex not specified) died during the study but the deaths could not be attributed to compound administration. Final mean body weights of the male mice and female mice were not statistically different from those of the controls except for the high-dose female group which showed statistically significant decreased body weights. A slight increase in the incidence of perivascular lymphoid hyperplasia and interstitial nephritis was reported in female mice given the two highest dose levels. No adverse effects were reported for male or female mice administered 140, 281, or 563 mg/kg bw/day of *dl*-2-isopropyl-5-methylcyclohexanol.

A carcinogenicity study was conducted in which groups of B6C3F1 mice of each sex were fed diets containing 0, 2,000 or 4,000 ppm *dl*-2-isopropyl-5-methylcyclohexanol

(approximately 0, 300, or 600 mg/kg bw/day, respectively) 103 weeks [National Cancer Institute, 1979]. Necropsies and histological examinations were performed on all animals at the termination of the study and on those found dead during the study. The mean body weights of the treated mice were slightly lower than those of controls. Survival of the treated male mice and low-dose female mice was similar to the vehicle control animals; however, survival of the high-dose group of female mice was significantly less than that of the control animals but was not accompanied by any evidence of toxicity. There was no evidence of neoplastic or nonneoplastic lesions of the male (penis, prepuce, preputial gland, prostate, or epididymis) or female (uterus, endometrium, or ovaries) reproductive system. An increase in the incidence of hepatocellular carcinomas was observed in high-dose male mice, but was not statistically different from that observed historically in mice of that age and strain (Haseman *et al*., 1986; no robust summary provided). A low incidence of alveolar/bronchiolar adenomas of the lung was observed in treated females but was not statistically different from the incidence of this neoplasm in historical control groups. Under the conditions of this study, the authors concluded that *dl*-2-isopropyl-5-methylcyclohexanol was not carcinogenic and did not produce any organ-specific toxicity for either sex of B6C3F1 mice at dose levels up to 600 mg/kg bw/day.

Fischer 344 rats were fed diets containing 0, 930, 1870, 3750, 7500, or 15,000 ppm *dl*-2-isopropyl-5-methylcyclohexanol (approximately 0, 93, 187, 375, 750 or 1500 mg *dl*-2-isopropyl-5-methylcyclohexanol/kg bw/day, respectively) for 13 weeks National Cancer Institute, 1979]. Necropsies were performed on all animals at the end of the study. Histopathological examination was performed on tissues from selected animals. Final mean body weights of the male and female rats at all dose levels were similar to those of the controls. A slight increase in the incidence of interstitial nephritis was observed in high-dose male rats. No adverse effects were reported for male or female rats administered up to 750 mg/kg bw/day of *dl*-2-isopropyl-5-methylcyclohexanol.

Fischer 344 rats of each sex were fed diets containing 0, 3,750, or 7,500 ppm *dl*-2-isopropyl-5-methylcyclohexanol (approximately 0, 187, or 375 mg *dl*-2-isopropyl-5-methylcyclohexanol/kg bw/day, respectively) for 103 weeks [National Cancer Institute, 1979]. Necropsies and histological examinations were performed on all animals at the

termination of the study and on those found dead during the study. The mean body weights of treated rats were slightly lower when compared to the controls. Microscopic examination of tissues of test animals failed to reveal any evidence of neoplastic or nonneoplastic lesions, including those of the male (*e.g.*, penis, scrotum, prostate, mammary gland, or epididymis) or female (uterus, vagina, mammary gland, endometrium, or ovaries) reproductive system. Survival of the treated rats was similar to the control animals. Chronic inflammation of the kidney observed in the dosed older males was not considered by the authors to be related to the administration of *dl*-2-isopropyl-5-methylcyclohexanol since the effect is commonly observed in aged male Fischer 344 rats. There was no increase in the incidence of neoplasms of dosed females compared to that of control animals. In treated females, fibroadenomas of the mammary glands occurred at a lower incidence than in the control group. Alveolar/bronchiolar adenomas or carcinomas were reported only for the female control rats. Under the conditions of this study, the authors concluded that *dl*-2-isopropyl-5-methylcyclohexanol was neither carcinogenic nor toxic for either sex of Fischer 344 rats at dose levels of up to 375 mg/kg bw of *dl*-2-isopropyl-5-methylcyclohexanol.

Since publication of the variety of subchronic and chronic studies on aliphatic and alicyclic ketones, the mechanism of action associated with the formation of *alpha*-2-microglobulin in male rats has been extensively studied. These research studies are applicable to any substance exhibiting *alpha*-2-microglobulin-type nephropathy. Therefore, no robust summaries have been prepared for the studies cited below.

It has been clearly demonstrated that renal lesions, which were also observed in numerous NTP studies, resulted from the accumulation of aggregates of *alpha*-2-microglobulin (a low molecular-weight protein synthesized in the liver) and test agents or its metabolites in the P2 segment of the renal proximal tubule. This phenomenon was initially observed in the male F344/N rat [Strasser *et al.*, 1988; Borghoff *et al.*, 1990] but has now been identified in other well-recognized strains of laboratory rats [Hildebrand *et al.*, 1997; Saito *et al.*, 1996].

The gene that encodes *alpha*-2-microglobulin has been isolated and the sequence deduced [Untermann *et al*., 1981]. These proteins are expressed in the liver under hormonal control [Roy and Neuhaus, 1967; Wang and Hodgetts, 1998]. *alpha*-2-Microglobulin belongs to the *alpha*-2-microglobulin super family of proteins that are characterized by a unique hydrophobic binding pocket. The lesions do not develop in the female rat or in humans [Bucher *et al*., 1986]. Subsequent investigations have shown that the *alpha*-2-microglobulin nephropathy found in the male rat does not develop in mammals that do not express the hepatic form of *alpha*-2-microglobulin [Swenberg *et al.*, 1989; Dietrich and Swenberg, 1991], mice [Bucher *et al*., 1986; Lehman-McKeeman and Caudill, 1994] and dogs [Webb *et al*., 1990].

Transgenic mice that express rat *alpha*-2-microglobulin were tested for their ability to form hyaline droplets and develop nephropathies similar to their adult male rat counterparts [Lehman-McKeeman and Caudill, 1994]. This study involved male rats as positive control, transgenic C57BL/6J mice as experimental group and native C57BL/6 mice as negative controls. The animals at age 70-75 days were placed in metabolic cages and received 150 mg/kg bw/day of *d*-limonene in corn oil by gavage for three days. Limonene is a potent inducer of renal nephropathy in adult male rats [Environmental Protectioin Agency, 1991; NTP, 1990]. Twenty-four (24) hours after the last dose the animals were sacrificed and the kidneys analyzed for evidence of nephropathy. Hyaline droplet formation was evaluated on a subjective scale, size and intensity (0-4) multiplied by tubular loading (0-3) for an overall scale of 0-12 with 12 being the most severe. In the absence of *d*-limonene the control groups transgenic mice and rats showed a hyaline droplet score of 1+/-0 and 6+/-0.5, respectively. The test transgenic mice and rats showed a hyaline droplet score of 2.5+/-0.3 and 11+/-1.3, respectively upon dosing with *d*-limonene. The native mice developed no signs of hyaline droplet formation and tested negative for presence of *alpha*-2-microglobulin in their urine. The authors assert that based on the data presented "*alpha*-2-microglobulin is the only protein that is involved in the etiology of hyaline droplet nephropathy".

An increase in the kidney-type-*alpha*-2 microglobulin was seen in male Sprague-Dawley rats when these animals were administered 200 mg/kg bw/day of isophorone by gavage

for 7 days. The increases in the urinary kidney-type- *alpha*-2-microglobulin are dose-dependent and parallel-elevated accumulation in the kidney cells [Saito *et al.*, 1996].

In another study, adult male Wistar rats were administered two groups of chemical compounds, including 138 mg/kg bw of isophorone, potassium bromate, 2-propanol and a series of benzene and anthracene derivatives, to study induction of accumulation of *alpha*-2-microglobulin and structure-activity relationships. A monoclonal antibody against *alpha*-2-microglobulin was employed in a competitive ELISA procedure to determine its concentration in urine or tissue samples without purification. Plasma concentrations of *alpha*-2-microglobulin were not significantly increased by any of the test compounds at 1 mmol/kg bw. Kidney tissue concentrations were found to be 297-300% higher than that of controls. The hyaline droplet accumulating (HDA) potential was dependent on the test compound but there was no relationship between HDA activity and the structure or the pathway used to metabolize the test substance [Hildebrand *et al*., 1997].

The above studies depend exclusively on histopathologic evidence to detect *alpha*-2-microglobulin nephropathy. An *in vitro* assay based on the prerequisite of a chemical or metabolite to *alpha*-2-microglobulin has been developed that predicts, in greater than 90% (22/24) of the substances tested, the ability to induce *alpha*-2-microglobulin nephropathy [Lehman-McKeeman and Caudill, 1999]. *d*-Limonene-1,2-epoxide is well characterized as an *alpha*-2-microglobulin nephropathy inducer and has a steady state binding constant (K_d) of 5 x 10^{-7} M [Lehman-McKeeman *et al.*, 1989]. Based on this, a competitive binding assay was developed with [^{14}C]-d-limonene-1,2-epoxide and male rat urinary protein concentrate. Homogenous *alpha*-2-microglobulin was obtained from adult male rats [Lehmann-McKeeman and Caudill, 1992]. The assay was run with three series of competitive inhibitors terpenes (5), decalin/decanes/decanones (10), and halobenzenes (8). Total male urinary protein was incubated for 1 hour with the test materials, ranging from 0.001 to 3000 microM, and 0.5 microM [^{14}C]-d-limonene-1,2-epoxide. The ability of the test materials to displace 50% of the radiolabelled limonene epoxide from the protein was evaluated and IC50 values were calculated. An IC50 value of less than or equal to 100 microM for the terpene and decalin/decanone series is

considered predictive of *alpha*-2-microglobulin droplet formation. 2-Decanone was predicted to exhibit *alpha*-2-microglobulin droplet activity in male rats. Substances with an IC50 calculated at higher than 100 microM in the competitive binding assay were subjected to microsomal oxidation to generate metabolites that would bind to *alpha*-2-microglobulin. Three of the halobenzenes 1,2-, 1,4-, and 1,3-dichlorobenzene tested positive for *alpha*-2-microglobulin binding when incubated in the presence of rat liver microsomes. Parallel *in vivo* tests were performed in rats and hyaline droplet formation in the kidney was assessed to confirm the *in vitro* results. The authors concluded that the *in vitro* assay is greater than 90% predictive of *alpha*-2-microglobulin nephropathy induction in male rats without being invasive or requiring additional animal testing [Lehman-McKeeman and Caudill, 1999].

To further investigate kidney tissue concentration of *alpha*-2-microglobulin in the lysosomal portion, intact kidney lysosomes were isolated from untreated or 2,2,4-trimethylpentane (TMP)-treated rats and their ability to take up *alpha*-2-microglobulin was compared. It was found that *alpha*-2-microglobulin could be directly taken up in the presence of the heat shock cognate protein (*hsc*73). Hsc73 contributes to the normal degradation, lysis, of *alpha*-2-microglobulin in rat kidney and liver. However, in the presence of a chemical (TMP) known to induce aggregation of *alpha*-2-microglobulin, the activity of this pathway is increased. This may be due to an increase in the concentration of a receptor protein in the lysosomal membrane, which accelerates the uptake of cytosolic protein, *alpha*-2-microglobulin [Cuervo *et al.*, 1999].

While humans produce low molecular weight serum proteins, which are reabsorbed by the kidney, there is no evidence that *alpha*-2-microglobulin is produced [Olson *et al.*, 1990]. Urine collected from adult male rats and humans revealed no evidence that *alpha*-2-microglobulin production occurs in humans [Olson *et al.*, 1990].

It is unknown whether any human serum proteins possess a binding site similar to that of *alpha*-2-microglobulin. Although this is a possibility, it appears remote, since female rats, mice, and dogs do not show the renal changes noted in male rats exposed to isophorone. It should be noted that there is a class of human proteins referred to as the *alpha*-2-

microglobulin related proteins. They appear to have no functional relationship to the adult male rat urine proteins. The human protein has a higher molecular weight, 25 kDa and is a component of a neutrophil gelatinase complex [Kjeldsen *et al.,* 2000; Triebel *et al*., 1992]. An extensive review of the current scientific literature and genome databases reveals no native protein or biological entity that acts as a nephropathy agent like mature male rat *alpha*-2-microglobulin. The accumulated evidence indicates that it is the unique anatomical, physiological, and biochemical properties of the male rat kidney, especially the proximal convoluted tubule, that allows isophorone to interfere with renal processing of the strain-specific *alpha*-2-microglobulin. Therefore, this process is not predictive of human carcinogenicity. In a comprehensive review of *alpha*-2-microglobulin nephropathy and associated renal tubule tumors produced in the male rat exposed to isophorone and other simple ketones and hydrocarbons (*e.g*., limonene, decalin and methyl isobutyl ketone), it was concluded that the F344/N male rat is not an appropriate model for assessing human renal carcinogenic risk [Environmental Protection Agency, 1991]. After careful review, it has been concluded that the mechanisms leading to the renal carcinogenic findings in the male rat are largely known and strongly indicate that the nephropathy associated with male rats have no significance for human risk assessment [Burdock *et al*., 1990].

Based on the results of these subchronic and chronic studies, it can be concluded that the renal pathology reported in male rats treated with the linear and branched-chain aliphatic and alicyclic ketones in the KB4 /KB3 mixtures is unrelated to the human health assessment. It can also be concluded that exposure to low levels of the ketones in KB4/KB3 provide no significant potential for toxicity, neurotoxicity or carcinogenic ity. Therefore, it is not necessary to conduct additional studies on constituents of the KB4/KB3 mixtures.

3.4.4 Reproductive Toxicity

In two separate OECD 421 reproductive/developmental screening studies (see developmental section below), groups of male or female Sprague-Dawley rats were exposed to atmospheres containing 0, 80, 400, or 1,000 ppm 2-heptanone or 1, 2.5 or 5.0

mg/L (214, 535, or 1070 ppm) of 5-methyl-2-hexanone 6 hours daily for either 50 or 34-47 days, respectively. In addition to measurement of maternal and reproductive parameters, epididymal spermatozoan numbers, sperm motility and testicular spermatid head counts were monitored. Male and females were evaluated for clinical signs, body weight gain and food consumption. Measurement of reproductive parameters revealed no evidence of reproductive toxicity even at the highest exposure level for either substance. Based on a decrease in body weight in the 2-heptanone study, the maternal NOAEL was concluded to be 80 ppm [Eastman Kodak Co., 1996, 2001b]. When these data are combined with the observation that there were no effects to reproductive organs in the subchronic or chronic studies on linear and branched-chain aliphatic and alicyclic ketones (see repeat dose studies) it is concluded that the aliphatic ketones in KB4 exhibit a low potential for reproductive toxicity.

A combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD No. 422 Guideline study), male and female CD rats (12/sex/group), age 8 weeks of age were given daily doses of 0, 100, 300, or 1000mg/kg of 2,6,8-trimethyl-4-nonanone (IBHK) by gavage in a corn oil suspension for 2 weeks prebreeding, two weeks breeding, and 10 days postbreeding for males and 2 weeks prebreeding, two weeks breeding, through gestation (3 weeks) and lactations (4 days). Clinical observations conducted on all rats before-exposure and weekly throughout the study and on mated females on gestation day (gd) 0, 7, 14 and 20, and lactation day (Id) 4 revealed increased salivation (padoral staining - clear) at all dose levels in both sexes. Salivation was transient, usually ending within one hour of dosing, suggesting a local response to the taste of the test material. Perineal urine soiling was increased in males in the 1000 mg IBHK/kg/day group. Regular measurement of body weight and food consumption revealed no significant differences between test and control groups.

Measurement of reproductive indices revealed no treatment-related effects at any dose level on any of the reproductive parameters, pup survival indices or sex ratio that were evaluated. Pup survival was significantly higher than controls on Id 1 in the 300 and 1000 mg/kg/day dose groups; however, this finding is attributed to the lower value in the control group and was not considered adverse. There was a small, statistically significant

decrease in gestation length of the 100 and 1000 mg/kg/day dose groups. However the value was within the range of the historical control data and not considered to be treatment-related.

Litter size and pup body weights showed decreases in pup body weights of male and female pups on pnd day 1 and 4 in the 1000 mg/kg/day dose group were initially identified as being statistically different from controls using an ANOVA, however, due to the increased litter size in the high-dose group, an ANCOVA was used to determine if the difference in pup body weight was due to litter size and not treatment. The ANCOVA indicated that the effects on pup body weight were not significant on pnd 1 but were significant on pnd 4.

Treatment did not affect total motor activity count in either sex. The distribution of the motor activity counts within a session were also not affected by treatment in males, however, was statistically different for the females. For interpretation of this statistically significant interaction, additional examination of the data was performed. All of the double interactions containing treatment, time or epoch were examined, indicating that there was a significant difference in time-epoch, but that it was due to a difference in days not treatment. The female data for overall count distributions for the four treatment groups displayed differences from baseline and post-treatment conditions, irrespective of treatment. Linear contrasts indicated that that triple interactions were not statistically significant and the p values do not support a dose-response relationship. The interpretation of these data led to the conclusion that the statistically significant triple interaction in female rats represents a difference between days rather than an effect of treatment.

Haematology revealed that males given 300 or 1000 mg/kg/day had haemoglobin levels that were slightly lower (statistically significant) than the controls. The differences were not considered treatment-related as they were within, or in close proximity to, the historical control range. Prothrombin time for males in the 1000 mg/kg/day dose group was higher than controls, statistically significant and outside of the historical control

range. Clinical chemistry determinations revealed serum cholesterol levels in males and females in the 1000 mg/kg/day group were statistically higher than the control and slightly outside of the historical control range. The differences were considered to possibly be treatment-related, but not toxicologically significant because increase compared to the historical control data (43-58 mg/dl for males and 42-73 mg/dl for females) was minor. The AST and total protein of males in the 1000 mg/kg/day group were higher and lower, respectively, than the control values and statistically identified. Alterations in AST were considered treatment-related, but secondary to the hepatocellular hypertrophy noted in the necropsy of these animals. ALP activity in the 1000 mg/kg/day females was lower than the control and historic values (66-97 u/I). While this was considered treatment related, it was not considered toxicologically significant due to the minor nature of the difference and lack of apparent adverse effects.

Dose-related increases in absolute and relative liver weights occurred in males and females given 100, 300 or 1000 mg/kg/day. Absolute and/or relative kidney weights of males and females given 300 or 1000 mg/kg/day were also increased. These differences were statistically identified and outside of the historical control data. Therefore, the differences were considered to be treatment-related. Absolute and/or relative thyroid weights of males given 100 or 1000 mg/kg/day and females given 300 or 1000 mg/kg/day were higher than control weights and were statistically identified, higher than historical control data and considered to be treatment-related. The relative weight of the ovaries of the females given 100 mg/kg/day was significantly increased as compared to controls and increased as compared to the historical data. However, this difference was not considered to be treatment-related given the normal reproductive performance of these females, lack of any histological changes, and the minor difference from controls. In addition, the absolute and relative ovarian weights from all dose levels in this study, including controls, were lower than historical controls.

There were no treatment-related gross pathologic observations.-Histopathology examinations revealed male rats given 100, 300 or 1000 mg/kg/day had degenerative kidney effects that were in excess of that observed in the control male rats and were

interpreted to be treatment related. Male rats given 1000 mg/kg/day had degenerative changes involving the renal tubules that were slight to moderate in severity compared with controls. This lesion primarily involved the proximal convoluted tubules and was characterized by an increase in cytoplasmic basophilia of tubular epithelial cells, thickening of the tubular basement membrane, presence of granular casts within the tubular lumens and the interstitial accumulation of mononuclear inflammatory cells. Necrotic tubular epithelial cells were also noted in the majority of males given the 1000 mg/kg/day were multifocal in distribution and very slight in severity. Male rats given 300 mg/kg/day had similar degenerative tubular changes of slight to moderate severity, and some of these rats had a very slight necrosis of tubular epithelial cells. Male rats given 0 or 100 rng/kg/day had a very slight tubular degeneration that was similar to the degeneration seen in the males of the higher dose groups. This slight degeneration occurred in 3 of 12 control males as compared to 7 of 12 males given 100 mg/kg/day. The increased incidence of this observation in males given 100 mg/kg/day was considered to be treatment-related. Degenerative kidney lesions were noted in females given 0 or 1000 mg/kg/day but were interpreted to be spontaneously occurring because of the low incidence and minimal severity. Male rats given 100, 300 or 1000 mg/kg/day also had eosinophilic staining (hyalin) cytoplasmic inclusions/droplets in the proximal tubules, which were infrequently observed in the control males, not observed in any of the females and was interpreted to be treatment-related. The results were consistent with, but not diagnostic for, alpha 2u globulin accumulation. Increased levels of this protein in the proximal convoluted tubular cells of male rats has been shown to cause tubular degeneration, although this is not considered relevant for human risk assessment as humans do not develop nephropathy due to differences in this protein. Males given >/= 100 mg/kg/day and females given >/= 300 mg/kg/day had treatment-related hypertrophy of hepatocytes in the liver. The effect was more prominent in males, given that it was seen even at the lowest dose point and based on the panlobular distribution within the hepatic Iobule. Males given *>/=100* mg/kg/day had treatment-related hypertrophy of follicular epithelial cells of the thyroid gland. Thyroid effects were not observed in female rats.

Based on te results of this study, a no-observed effect level (NOEL) for general toxicity could not be determined for male rats based on the nephrotoxic effects at the lowest dose level, while the NOAEL for general toxicity in females was 100 mg/kg/day. The NOEL for reproductive effects was 300 mg/kg/day. The NOEL for neurological effects was 1000 mg/kg/day, the highest dose level tested (Dow Chemical, 2002).

In a screening assay for fertility activity, groups of 8 female CF1 mice were given 50 mg/kg bw dose of 2-pentadecanone, 8-pentadecanone, or 2-undecanone by intraperitioneal injection daily during gestation. Diethylstilbesterol was administered as a positive control (10 ug/kg bw). Dams were observed for signs of toxicity and body weights were recorded during gestation. The percent pregnant, number of viable fetuses per litter, number of resorption sites, and dead in utero per litter were recorded and expressed as a percent of the control value. No effects on maternal body weight were observed and no sign of toxicity were reported. For the test groups compared to the control group, pregnancy rate was in the range of 50-100%, the average number of resortion sites per litter was 0% and the average number of fetuses per litter was in the range of 60-81%. Diethylstilbesterol was used as a positive control (10 ug/kg bw). The positive control showed 0% pregnancy rate, 0% fetuses per litter, and 0% resorption sites per litter. Under conditions of the experiment, a 50 mg/kg bw dose of 8-or 2-pentadecanone or 2-undecanone given daily by intraperitoneal injection to female rats produced no maternal and no or mild reproductive effects [Carlson *et al.*, 1975].

Virgin Crl CD rats were administered oral dose levels of 0, 150, 750, or 1,500 mg/kg bw/day of a mixture of alkyl-substituted cyclohexanones and cyclohexanols used in the 28-day study above [Serota, 1990] by gavage for 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days [Hoberman, 1989]. The composition of the test material was identical to that used in the 28-day study [Vollmuth, 1990, no robust summary provided]. The study design included measurement of parameters for reproductive and developmental toxicity. Maternal indices monitored included twice-daily clinical observation, measurement of body weights, food consumption, duration of

gestation, and fertility parameters (mating and fertility index, gestation index, and number of offspring per litter). Offspring indices monitored included daily observation, clinical signs, examination for gross external malformations, and measurement of mortality (number of stillborns), viability (pups dying on days 1-4), body weight and body weight gain.

At the two highest dose levels, maternal mortality was increased, significant decreases in maternal body weight and food consumption were reported, clinical observations of the dams included decreased motor activity, ataxia, dysnea, rales, chromorrhinorrhea, un-groomed coat and thin appearance, and significant increases in pup mortality were reported. Live litters were reported for 9/19, 8/10, 5/6, and 1/4 pregnant females in the control, 150, 750, and 1,500 mg/kg bw/day groups, respectively. Increased number of dams with stillborn pups, stillborn pups, and late resorptions *in utero* were reported in the mid-dose group. At the highest dose, 2 rats had only resorptions *in utero* when found dead on gestation day 23 and one rat possessed only empty implantation sites *in utero* on day 25 of presumed gestation. Even at the highest dose level, there was no evidence of an effect of the test article on implantation, duration of gestation, pup sex ratio, or gross morphology of pups. Based on these results the authors concluded that the maternal NOAEL for reproductive effects was 150 mg/kg bw/day and the offspring NOAEL for developmental effects is greater than 150 mg/kg bw/day, but less than 750 mg/kg bw/day.

In a dominant lethal assay, males rats were gavaged with up to 3,000 mg/kg bw of 2-isopropyl-5-methylcyclohexanol as a single exposure or up to 1,150 mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol for 5 days [Food and Drug Administration, 1975]. Male rats were mated with 2 female rats per week for 7-8 weeks following the last treatment. Fourteen days after mating, females were killed and the uterus was examined for early deaths, late fetal deaths, and total implantations. No effect on early deaths, late fetal deaths and total implantations was reported when 2-isopropyl-5-methylcyclohexanol was administered to male rats prior to mating.

Given the lack of any significant reproductive effects in the reproductive/developmental screening studies and the absence of any significant effects to the reproductive organs of

animals in subchronic and chronic repeat dose studies, it is concluded tha t aliphatic ketones and alkyl-substituted cyclohexanones exhibits a very low order of reproductive toxicity. No additional testing is recommended for the KB4/KB3 mixture.

3.4.5 Developmental Toxicity

Groups (8/group) of sexually mature adult female CD rats of age 10-11 weeks and weight 200-250g were given doses of 0, 250, 500, 750 and 1000 mg IBHK/kg/day in a 0.5% METHOCEL suspension by gavage daily for days 6-20 of gestation. During the study clinical observations were conducted daily and maternal body weights and food consumption were recorded on GD 0 (at the supplier), 3, 6, 9, 12, 15, 18 and 21. At conclusion of the study, all animals were submitted for a complete necropsy on day 21. Weights of the liver and kidneys were recorded and organ to body ratios calculated. Sections of liver, kidneys and gross lesions were preserved. An examination of the uterus for the number of implantation sites and resorptions, and the ovaries for the number corpora lutea was performed. The position and number of resorptions and normally developing fetuses were recorded. Corpora lutea for non-pregnant animals were not counted. The uteri of animals lacking visual implantations were stained and examined for evidence of early resorptions to verify pregnancy status.

Although there were isolated clinical sign that were mainly transient in nature, these were no consistent evidence of clinical changes related to administration of the test substance. Exposure to 750 or 1000 rng IBHK/kg/day resulted in a decrease in body weight gains, 10 and 11% respectively, although these decrease were not significant. There were no significant differences in feed consumption between control and test animals. Doses of 750 and 1000 mg/kg/day produced statistically significant increases in absolute (28 and 33%) and relative liver weights (30 and 33%). Relative liver weight was also significantly increased at 500 mg/kg/day (16%). There was a 14% increase in absolute liver weight at 500 mg/kg/day that was considered treatment-related, but not statistically significant. Mean relative kidney weight was significantly increased by 19% and absolute kidney weight was increased by 15%, not statistically significant, at the 1000 mg/kg/day

dose level. There were no treatment-related gross pathologic observations.

Minimal reproductive and embryo-fetal effects occurred only at the highest dose level. There were no significant treatment-related effects on pregnancy rates, number ol corpora lutea, implantations, resorptions per litter with resorptions, or litter size at any dose level. Mean percent post-implantation loss was significantly increased at 1000 mg/kg/day and number of resorptions per litter was significantly increased at 1000 mg/kg/day, but the increase was not statistically significant and there was not a related decrease in the number of viable fetuses as compared to control. A significant decrease in mean percentage pre-implantation loss was seen at 1000 mg/kg/day, but a decrease in this parameter is not considered adverse. There were no treatment-related changes in pup clinical signs, weight gain, or abnormalities compared to controls at any of the test concentrations. Based on the above observations, the no-observed-adverse-effect level (NOAEL) for maternal toxicity was 250 mg/kg/day while 1000 mg/kg/day was considered a no-observed-effect-level (NOEL) for embryo/fetal toxicity

Groups of pregnant LAK:LVG(SYR) hamsters were given 0, 96, or 960 mg/kg bw of 6,10-dimethyl-2-undecatrienone dissolved in acetone (5%) and solubilized in Tween 20 by gavage on day 8 of pregnancy. The low- mid-, and high-dose group contained 6, 9, and 14 animals. The doses were chosen based on the median effective dose of retinoids that induce terata (ED50) in hamsters. Animals were sacrificed on Day 14 and average fetal and maternal body weight were measured. Developmental parameters monitored included, number of litters, abnormal litters, implantation sites, number of resorptions, number of abnormal live fetuses, number dead fetuses, mean litter frequency, and characterization of malformations. The only effect reported was a significant reduction in maternal weight gain in the 960 mg/kg bw group. The authors concluded that dose levels up to and including 960 mg/kg bw failed to show any evidence of developmental toxicity in golden Syrian hamsters. Based on the depressed body weights of females at 960 mg/kg, the dose level of 96 mg/kg was concluded to be the NOAEL for maternal toxicity [Willhite, 1986].

Two OECD 421 reproductive/developmental screening studies have been performed on a straight-chain (2-heptanone) and a branched-chain (5-methyl-2-hexanone) ketone. Groups of male or female Sprague-Dawley rats were exposed to atmospheres containing 0, 80, 400, or 1000 ppm 2-heptanone 6 hours daily for either 50 or 34-47 days, respectively. Male and females were monitored for clinical signs, body weight gain and food consumption. All adult animals survived to study termination and there were no test substance-related changes in mean terminal body weight. For the 1000 ppm male group, there was a reduction in food consumption during days 0-7. Otherwise, there were no other differences in mean body weight, body weight gain, food consumption or food utilization among the groups throughout the study. Except for minimal reductions in activity level observed in the 400 and 1000 ppm groups during each exposure, no other test substance-related clinical abnormalities were noted. Mean sperm motility and mean epididymal spermatozoan and testicular spermatid counts were comparable among test and control groups. No test substance-related gross pathology was observed for adult animals from any group. No exposure-related changes were observed during histological examination of the reproductive organs of any of the test groups. There were no changes in pup clinical signs or body weight of the test groups compared to the controls. There were no abnormalities of the skeletal or tissues that could be related to administration of the test substance. Based on these data the no observable adverse effect concentration (NOAEC) for parental toxicity was concluded to be 80 ppm and the NOAEC for fetotoxicity was concluded to be 1000 ppm, the highest concentration tested [Eastman Kodak Co., 1996].

In the second study, groups of Sprague Dawley rats were exposed to 0, 1.0, 2.5, or 5.0 mg/L of 5-methyl-2-hexanone for 51(males) or 35-41 days (females). There was no evidence of parental, reproductive or fetal toxicity at any dose level tested. The NOEC for either toxicity endpoint exceeded 5.0 mg/L [Eastman Kodak Co., 2001b].

In an inhalation teratology study, groups of female Fischer F344 rats and CD-1 mice (22/group) were exposed to atmospheres containing 0, 25, 50, or 110 ppm of 3,5,5-trimethyl-2-cyclohexenone 6 hours daily during days 6 to 15 of gestation. There were no significant changes in the clinical signs, body weights, food consumption, or gross

evaluation at necropsy for the test and control groups of dams of either species. Measurement of live and dead fetuses and number of early and late resorptions and evaluation of implantation sites and Corpora lutea revealed no significant differences between test and control groups. There were no skeletal malformations or ossification variations that were considered related to exposure to the test substance for either species. The NOEC for Fisher F344 female rats and CD-1 female mice was reported to be 1,150 ppm [Traul, 1984].

Based on the interconvertability of alicyclic/aliphatic ketones and alcohols *in vivo*, data on the corresponding alcohol is also considered relevant to the teratogenic potential of the corresponding ketone. Teratology studies in four animal species were performed under Food and Drug Administration contracts for the isomer of 2-isopropyl-5-methylcyclohexanol. Studies in mice [Morgareidge, 1973a], rats [Morgareidge, 1973b], and hamsters [Morgareidge, 1973c] were performed using the same study design. In each study, virgin adult females (CD-1 outbred mice, Wistar rats, or golden hamsters) were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on day 6 and continuing daily through day 15 (mice and rats) or day 10 (for hamsters) of gestation, groups (22-23 for mice, 22-25 for rats and 19 to 23 for hamsters) of pregnant females were given 2-isopropyl-5-methylcyclohexanol by gavage in corn oil. Mice received 0, 1.85, 8.59, 39.9, or 185 mg/kg bw/day, rats received 2.18, 10.15, 47.05, or 218 mg/kg bw/day, and hamsters received 0.05, 21.15, 98.2, or 405 mg/kg bw/day. Negative control groups received corn oil by gavage daily while positive control groups received aspirin. On day 17(mice), 20 (rats), or 14 (hamsters), all dams were subjected to Caesarian section and the number of live litters, implantation sites, number of resorptions, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, implant sites per dam, percent of live and percent partial live resorptions, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10 times magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects. No effects on these parameters were reported in any of the species tested and the authors

concluded that there was no evidence of maternal or developmental toxicity at dose levels up to and including 185 (mice), 218 (rats), and 405 (hamsters) mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol during gestation.

Virgin adult female rabbits were artificially inseminated and beginning on gestation day 6 and continuing daily through day 18, pregnant rabbits were given 0, 4.25, 19.75, 91.7, or 425 mg/kg bw of 2-isopropyl-5-methylcyclohexanol by gavage in corn oil [Morgareidge, 1973d]. A positive control group received 2.5 mg/kg bw/day of 6-aminonicotinamide. On gestation day 29 all dams were subjected to Caesarian section and the number of *corpora lutea*, implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were recorded. The urogenital tract of each dam was examined for anatomical abnormalities. All live fetuses were placed in an incubator for 24 hours and evaluated for survival. All surviving pups were sacrificed and subjected to detailed visceral examination at 10 times magnification. All fetuses were cleared with KOH, stained with alizarin red S dye, and examined for skeletal defects. As reported for the 3 other species, there was no evidence of either maternal toxicity or developmental toxicity at dose levels up to and including 425 mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol. Given the results of this multiple species study, alkyl-substituted cyclohexanol derivatives exhibit a low potential for developmental toxicity.

In summary, the developmental toxicity testing for three aliphatic linear or branched-chain ketones and for two cyclohexanone derivatives indicated that constituents of the KB4/KB3 mixtures exhibit a low potential for developmental toxicity.

3.4.6 New Testing Required

No further testing is required.

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